THERAPY STUDIES IN MULTIPLE MYELOMA

O. A. van Dobbenburgh

THERAPY STUDIES IN MULTIPLE MYELOMA

Het uitkomen van dit proefschrift werd mede mogelijk gemaakt door financiële steun van de Firma Eli Lilly.

Stellingen

behorende bij het proefschrift van O. A. van Dobbenburgh
Therapy Studies in Multiple Myeloma, 26 september 1984

1. De Durie/Salmon-stagering van het multipel myeloom is nuttig voor het groeperen van patiënten, bijvoorbeeld in het kader van een prospectief therapie-onderzoek; voor het inschatten van de overleving van een individuele patiënt is zij echter minder geschikt.
2. De celkinetische eigenschappen van het multipel myeloom die de laatste jaren bekend zijn geworden hebben nog niet tot een verbetering van de behandeling van deze ziekte geleid.
3. Een patiënt met een onbegrepen polyneuropathie dient onderzocht te worden op de aanwezigheid van een paraproteïne.
4. Bij de behandeling van een tumor-hypercalciaemie met intraveneuze rehydratie is toediening van furosemide slechts dan geïndiceerd wanneer de rehydratie leidt tot symptomatische overvulling.
5. Bij een Kahler-patiënt met een asymptomatische hypercalciaemie dient rekening te worden gehouden met de mogelijkheid van verhoogde calciumbinding door het paraproteïne.
6. Bij Kahler-patiënten is het routinematig verrichten van isotopenonderzoek van het skelet niet geïndiceerd.
7. Tijdens de intraveneuze toediening van N-acetylcysteine ter behandeling van paracetamolvergiftiging moet men bedacht zijn op anafylactoide reacties.
8. Profylactische positieve eind-expiratoire druk ('PEEP') beademing van patiënten at-risk voor een adult respiratoir distress syndroom kan de ontwikkeling van dit syndroom niet voorkomen.
9. Tractus digestivus-bloedingen ten gevolge van medicamenten (corticosteroiden, antiflogistica) zijn zeer goed te behandelen met intraveneus toegediend somatostatine.
10. Besprekingen over reductie van kernwapens zijn belangrijk, maar zij gaan pas werkelijk betekenis krijgen indien tegelijkertijd de afschaffing van 'conventionele' wapens ter sprake komt.
11. Beperking van het aantal opleidingsplaatsen voor medisch specialisten: als het kalf verdronken is dempt men de put.
12. Een poes vervult in een gezin met jonge kinderen de rol van kat-alysator; het ziek worden of overlijden van die poes is meestal een kat-astrofe.

RIJKSUNIVERSITEIT TE GRONINGEN

THERAPY STUDIES IN MULTIPLE MYELOMA

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
in het openbaar te verdedigen op woensdag 26 september 1984
des namiddags te 2.45 uur precies

door

OTTO ARIJ VAN DOBBENBURGH
geboren te Halfweg

1984

Promotores:
Dr. B. Houwen, associate Professor of Haematology
Prof. Dr. M. R. Halie
Prof. Dr. H. O. Nieweg, F.R.C.P.

Referent:
Dr. J. Marrink

Aan Paula,
Aan Irene, Wouter en Harald

Voorwoord

Het onderzoek waarop dit proefschrift is gebaseerd werd verricht binnen de afdeling Haematologie (Hoofd: Prof. Dr. H. O. Nieweg) van de kliniek voor Inwendige Geneeskunde (Hoogleraar-Directeur: Prof. Dr. E. Mandema, Chef de Clinique: Prof. Dr. J. Trip) te Groningen.

Prof. Dr. E. Mandema en Prof. Drs. J. Scholten (tot mei 1984 Chef de Clinique) dank ik voor mijn opleiding tot algemeen internist.

Zeer veel dank ben ik verschuldigd aan Prof. Dr. Berend Houwen, die mij niet alleen introduceerde in en enthousiast maakte voor de haematologie, maar die tevens met veel energie en deskundigheid de basis legde voor dit proefschrift.

Prof. Dr. Ruud Halie nam na het vertrek van Berend Houwen naar Canada de begeleiding op zich en ik ben hem zeer erkentelijk voor onze stimulerende discussies en zijn steun in de organisatie rond deze dissertatie.

Prof. Dr. H. O. Nieweg dank ik voor de gastvrijheid die ik bij de afdeling haematologie geniet en voor de inzet waarmee mijn manuscripten steeds werden beoordeeld.

Dr. Jan Marrink en Dr. Theo Ockhuizen (afdeling Klinische Immunochemie) dank ik voor de waardevolle kritieken en suggesties en voor de supervisie over de vele immuunglobuline- en β2-microglobuline bepalingen.

De samenwerking met Drs. Henny Jurjens en Jaap Bijzet (afdeling Nucleaire Geneeskunde, Hoofd: Prof. Dr. M. G. Woldring), die de spermidine-radioimmunoassay ontwikkelden, heb ik steeds als zeer aangenaam ervaren.

Dr. Sjoerd Rodenhuis (thans: Antonie van Leeuwenhoek Huis) ben ik zeer erkentelijk voor de grote hoeveelheid energie die hij in het β2-microglobuline hoofdstuk heeft gestoken.

De tekeningen werden verzorgd door de heer J. Brouwer, terwijl Bert Schaalma en Els Muskiet-Stuut (bibliotheek) behulpzaam waren bij het verzamelen van de literatuur. De tabellen werden verzorgd door Ankie Bouma.

Verder dank ik alle collegae, verpleegkundigen, leden van de medische registratie en anderen die direct of indirect aan het tot stand komen van dit proefschrift hebben meegewerkt.

Zonder de continue steun en aanmoediging van Paula zou dit proefschrift wel zijn begonnen maar nooit zijn beëindigd.

Contents

Chapter 2 is based on the following publications:

B. Houwen, Th. Ockhuizen, J. Marrink and H. O. Nieweg.
Vindesine therapy in melphalan-resistant multiple myeloma.
Eur J Cancer 1980, 17, 227-232.

O. A. van Dobbenburgh, B. Houwen, M. R. Halie, J. Marrink, Th. Ockhuizen and H. O. Nieweg.
Progress report on vindesine treatment of melphalan-resistant multiple myeloma.
Eur J Cancer Clin Oncol 1983, 19, 861-862

Chapters 3-6 are published as a whole or in part in:

O. A. van Dobbenburgh, B. Houwen, H. Piersma, J. Marrink, Th. Ockhuizen, M. R. Halie and H. O. Nieweg.
Cyclophosphamide, doxorubicin, prednisone and vindesine combination chemotherapy in melphalan-resistant multiple myeloma.
Neth J Med 1984, 27, 25-30.

O. A. van Dobbenburgh, S. Rodenhuis, Th. Ockhuizen, E. Weltevreden, B. Houwen, V. Fidler, S. Meijer and J. Marrink.
β2-microglobulin in multiple myeloma: limited prognostic value and minimal correlation with tumour mass.
Submitted

O. A. van Dobbenburgh, M. R. Halie, H. Piersma, J. Marrink, Th. Ockhuizen and H. O. Nieweg.
Multiple myeloma with high tumour mass. Treatment with high-dose methylprednisolone, cyclophosphamide and vindesine.
Europ J Cancer Clin Oncol 1984, 20, 437-439.

O. A. van Dobbenburgh, B. Houwen, H. Jurjens, J. Marrink, H. O. Nieweg.
Plasma spermidine concentrations as early indication of response to therapy in human myeloma.
J Clin Pathol 1983, 36, 804-807.

Chapter 1

Introduction

This thesis describes some aspects of multiple myeloma with emphasis on the treatment and prognosis of the disease. In this chapter a review of the literature is presented concerning history, clinical aspects, diagnosis, staging, treatment, prediction of prognosis and cytokinetics. The differential diagnosis of a monoclonal immunoglobulin will be discussed.

1.1 History

In August 1844 Mr. Thomas Alexander McBean, then 45 years old, consulted his physician, Dr. Thomas Watson. His complaint consisted of severe pain in the chest which had appeared quite suddenly after a minor trauma. This pain persisted for several months, but disappeared eventually after bedrest and the application of a plaster cast. After a period of relatively good health skeletal pains returned in August 1845 never again to disappear. Mr. McBean became cachectic and died on the first of January 1846. On October 30, 1845, Dr. William MacIntyre, a physician to the Metropolitan Convalescent Institution, saw Mr. McBean in consultation and he examined the urine personally. He observed that the urine was of a very high specific gravity. Furthermore he noticed that the urine was slightly opaque at boiling temperature. When nitric acid was added to the boiling urine, it became clear, but showed a precipitate at cooling, which dissolved again when the urine was heated. He sent the urine for further investigation to Dr. Henry Bence Jones, a physician at the St. George's Hospital in London. Dr. Bence Jones confirmed the findings of Dr. MacIntyre and added the observation that protein also precipitated when the urine was boiled at 40-58 °C at a weakly acid reaction [1,2]. He spent many years of research in unravelling the chemical structure, which is why protein in urine which exhibits the four properties mentioned above, is still today called 'Bence Jones-protein'.

In 1873, von Rustizky, a Russian physician working in Recklinghausen's laboratory described a patient who had an expansive growing tumour of the temporal bone. At autopsy eight separate tumours

were found in the bone marrow and von Rustizky called them multiple myeloma's [3]. In 1883 Kühne, a physiologist at Amsterdam and a pupil of Claude Bernard, published a patient with a Bence Jones proteinuria [4]. This patient suffered from a paraplegia due to a skeletal disease which was diagnosed as 'acute osteomalacia'.

From 1879 to 1887 Dr. Otto Kahler in Prague treated a patient – an obstetrician – who suffered from severe skeletal pains and ultimately many spontaneous fractures. The urine of this patient showed the same properties as the previous two patients while autopsy disclosed extreme osteoporosis caused by masses of large proliferating cells, which Kahler called myeloma. He then concluded that: 'the marked 'albumosuria' must be a frequent symptom of this bone marrow disease, i.e. myeloma' [5]. Hence today the eponym Kahler's Disease is used, although the Russian literature favours the name Rustizky's Disease.

In 1900 Wright described the similarity existing between tissue plasma cells and the proliferating cells in the marrow of patients with multiple myeloma [6]. The possibility of obtaining bone marrow by sternal puncture (discovered in 1929, [7]) facilitated the diagnosis of myelomatosis and also led to recognition of an increasing number of patients having this disease. Further steps in the progress of myeloma research were the discovery of an increase in the serum protein level (1928), the electrophoresis analysis which permitted separation of the homogenous globulin fraction, and finally immunoelectrophoresis, enabling identification of specific light and heavy chains of the immunoglobulin molecules. Some historic aspects of the treatment of multiple myeloma will be mentioned in the section on the treatment (paragraph 1.6.1).

1.2 Differential diagnosis of a monoclonal immunoglobulin

Multiple myeloma is one of the so-called plasma cell dyscrasias. A striking feature of these diseases is the presence of a monoclonal immunoglobulin (paraprotein or m-protein). By means of immunoelectrophoresis the heavy chain indicating the immunoglobulin class (G,A,M,D or E) and the light chain (kappa or lambda) of the m-protein can be determined. In twenty percent of the myeloma patients the plasma cell clone produces only a light chain (so-called Bence Jones myelomas).

A patient who presents with an m-protein, does not always necessarily suffer from multiple myeloma. The differential diagnosis of the presence of a m-protein will be discussed briefly (see Table 1):

Table 1. Differential diagnosis of a monoclonal immunoglobulin.

Monoclonal Gammopathy of Undetermined Significance (MGUS)
Multiple Myeloma
Localized Plasmocytoma
Waldenström's Macroglobulinaemia
POEMS-syndrome
Heavy-chain Disease
Primary Amyloidosis

a) Monoclonal Gammopathy of Undetermined Significance (MGUS, also called benign monoclonal gammopathy).
This is the most common plasma cell dyscrasia. Of 574 m-proteins evaluated in 1980 (Mayo Clinic, Rochester, Minnesota) 72% were MGUS [8]. In a retrospective analysis at the University Hospital Groningen 104 (36%) out of 288 patients with a m-protein had MGUS [9]. In a study conducted at the Mayo Clinic 44 of 241 patients with MGUS eventually developed myeloma, macroglobulinaemia, amyloidosis or a lymphoproliferative disease [10].

The term 'benign monoclonal gammopathy' is therefore misleading. It follows that a patient with a MGUS should be kept in follow-up indefinitely. Patients with a MGUS are characterized by a serum m-protein level frequently less than 20 g/l (almost always less than 30 g/l), and the absence of anaemia, osteolytic bone lesions and significant Bence Jones proteinuria. Bone marrow plasmacytosis is less than 5%. The m-protein involved is either IgG, IgA or IgM [10]. Recently the occurrence of patients with 'Bence Jones' MGUS has been observed, but this is extremely rare [11].

The distinction of multiple myeloma from MGUS may at times be difficult. One of the most reliable criteria for a MGUS is a stable m-protein level during follow-up (see also [Diagnosis], paragraph 1.4). Suppression of polyclonal immunoglobulins is not a reliable feature for distinguishing between MGUS and multiple myeloma [12,13]. It should be mentioned that a m-protein may be encountered in the presence of other lymphocytic and also non-lymphocytic diseases such as diffuse non-Hodgkin lymphoma, auto-immune disease and Gaucher's disease [14,15,16,17]. A causal significance of these disorders for the development of the m-protein has been suggested but not proved.

b) Multiple myeloma (discussed in paragraph 1.3 to 1.8)

c) Localized plasmocytoma.
This entity (related to multiple myeloma) can be devided into two

categories: solitary myeloma and extramedullary plasmocytoma. Together they form 10% or less of the malignant plasma cell dyscrasias. Twenty-five percent of patients with a localized plasmocytoma shows a serum m-protein. This m-protein level is generally less than 15 g/l. Solitary myeloma mostly presents as an osteolytic lesion in the skeleton causing pain. Bone marrow examination and complete blood counts are normal. Therapy consists of local surgery and/or radiation. Within a period of ten years after local therapy, 85% of the patients shows a local recurrence, a new solitary myeloma elsewhere or true multiple myeloma [18,19]. Eighty-five percent of the patients with an extramedullary plasmacytoma presents with a localisation in the head or upper-respiratory tract. The most frequent sites are the maxillary sinuses, the rhinopharynx, the nasal and tonsillary fossae and the larynx [20,21].

d) Waldenström's macroglobulinaemia.
This disease is characterized by the prescence of an IgM m-protein and a bone marrow infiltration with mature looking lymphocytes. Differentiation between Waldenström's disease and multiple myeloma is not difficult. Some rare patients with a myeloma producing an IgM m-protein have been described [22].

e) POEMS syndrome.
This rare syndrome is characterized by a peripheral Polyneuropathy, Organomegaly (hepatosplenomegaly), Endocrinopathy (hypothyroidism, sex hormone dysregulation and gynaecomastia), M-protein and Skin changes (pigmentation). The underlying plasma cell dyscrasia can either be multiple myeloma, solitary myeloma or MGUS. The skeletal lesions are frequently osteosclerotic in contrast to 'normal' myeloma. The clinical picture is dominated by the neuropathy. Nerve biopsies show demyelinization, axonal degeneration or both. Patients with solitary myeloma have the best prognosis with curative local therapy, which frequently results in disappearance of the neuropathy [23,24].

f) Heavy chain disease.
Patients with this disease have characteristically a m-protein which consists of (a part) of the heavy chain of the immunoglobulin. Three different types can be identified: alpha-heavy chain disease (IgA heavy chain) characterized by malabsorption, steatorrhoea and lymphoid-plasmacytoid infiltration of the gut lamina propria; mu-heavy chain disease (IgM) usually associated with chronic lymphocytic leukaemia and large amounts of Bence Jones protein in the urine; gamma-heavy chain disease (monoclonal production of the Fc part of the IgG

molecule) with fatigue, weakness, fever and lymphadenopathy as presenting symptoms. Histologic findings range from an increase in plasma cells and lymphocytes in lymph nodes and bone marrow to classical malignant lymphoma [25].

g) Primary amyloidosis.
This disease – separated from myeloma associated amyloidosis by the absence of the diagnostic features of multiple myeloma – is characterized by weakness, fatigue, loss of weight, ankle edema, dyspnoea, paraesthesias and light-headedness or syncope as presenting symptoms. Frequently hepatomegaly and macroglossia are noted, as well as purpura. Other manifestations are congestive heart failure, nephrotic syndrome and carpal tunnel syndrome. The amyloid fibrils consist of light chains. In 88% of the patients a m-protein can be found in the serum or in the urine [26].

1.3 Symptomatology

Multiple myeloma is a disease characterized by proliferating plasma cells. This proliferation mainly takes place in the blood forming bone marrow, thereby affecting the bone tissue and the bone marrow itself. These plasma cells generally produce a monoclonal immunoglobulin. A major part of the symptoms of myeloma can at least partly be explained by these properties.

1.3.1 Skeletal involvement

The involvement of bone tissue results in several symptoms: skeletal pains, pathological fractures and hypercalcaemia. Radiologic examination may disclose asymptomatic 'punched-out' bone lesions (especially of the skull) and/or diffuse osteopenia (especially of the vertebrae). For many years the skeletal pathology was described directly to the expansive growth of the plasma cells, but in 1972 a substance was isolated from stimulated B and T lymphocytes, which was able to stimulate osteoclastic activity. This substance was called osteoclast activating factor (OAF) [27]. This OAF has also been isolated from cultured myeloma cells [28]. Although OAF is present in serum of myeloma patients [29], the bone tissue of bones not invaded by plasma cells is generally normal [30]. This presumably indicates that OAF-effects are concentration dependent. The problem of osteolysis in myeloma has not been elucidated completely. This is indicated by the finding of low OAF-serum levels in patients with extreme skeletal changes on one hand and the finding of raised OAF-levels in patients

with a reactive plasmocytosis on the other [31]. Furthermore, the role of the prostaglandin system in myeloma-associated osteolysis has not been clearly outlined. The use of indomethacin is very effective in treatment of skeletal pains but also appears to lower serum OAF-levels. It has been suggested that prostaglandins play a role in the production of OAF [31,32,33].

Many studies have shown that conventional radiography is a more sensitive method for skeletal evaluation in myeloma patients than isotope bone scans [34,35]. This is caused by the absence of bone repair in areas of bone destruction which correlates with the frequently found normal serum alkaline phosphatase (a parameter of osteoblastic activity). Fractures on the other hand, frequently cause a transient rise of alkaline phosphatase and isotope bone scans are positive in the areas of recent fractures.

1.3.2 Bone marrow involvement

A further aspect of bone marrow infiltration by plasma cells is the replacement of the normal blood forming elements. This results in anaemia causing fatigue and malaise, trombocytopenia and granulocytopenia. Anaemia is more frequently encountered than trombocytopenia and granulocytopenia. The latter two -if not due to chemotherapy- indicate a very severe bone marrow invasion.

1.3.3 Consequences of a monoclonal immunoglobulin

The presence of a monoclonal immunoglobulin (paraprotein or m-protein) is encountered in the majority of patients. The so-called non-secretory myelomas – the plasma cells possess an intracytoplasmatic immunoglobulin, but do not secrete it – constitute 1-4% of patient populations in several large studies [36,37]. Non-synthesizing myeloma – in which immunofluorescence studies fail to disclose intracytoplasmatic immunoglobulins – is extremely rare [38].

Approximately 75% of the patients shows the production of an intact immunoglobulin and 15-20% shows the production of only a monoclonal light chain (Bence Jones myelomas). The distribution frequency of the various m-proteins in several large patient populations is shown in Table 2.

In contrast to Waldenström's macroglobulinaemia the symptomatic hyperviscosity syndrome is only rarely found in myeloma patients. High serum m-protein concentrations usually cause an increase in serum viscosity in the laboratory but clinical symptoms are rare. When a hyperviscosity syndrome in myeloma is diagnosed, the m-protein will very likely be found to be IgA or IgG_3 [43,44] since both

Table 2. Distribution of monoclonal immunoglobulins in multiple myeloma.

	USA 1963 (39[a])	UK 1968 (36)	Australia 1969 (40, 41)	Groningen 1979 (42)	USA 1980 (12)
IgG	54[b]	53	58	63	56
IgA	23	25	29	22	22
Light-chain	22	19	12	14	18
IgD	–	1	1–2	–	2
No M-protein	2	–	–	1	0.3
Biclonal	–	2	–	–	2
Total no. of patients	262	212	202	107	940

a. reference number; b. percent of total patient number.

immunoglobulins have a tendency to form polymers. M-proteins sometimes behave as cryoglobulins (5-6% in large series) but the actual occurrence of symptoms e.g. Raynaud phenomenon, is rare [45].

The monoclonal globulins per se do not cause further symptoms, however the free light chains in Bence Jones myelomas and the light chains in 'whole-immunoglobulin myelomas' (in which production of heavy and light chain is unbalanced) are extremely important because of their adverse effects on renal function. A decrease in renal function secondary to myeloma is not a 'symptom' in the strict sense.

However, it is frequently encountered in multiple myeloma: at the moment of diagnosis 20% of the patients have a raised serum creatinine level [46,47]. In end-stage disease renal function is strongly impaired in 70% of the patients [47]. In the same study renal failure was ranking second as cause of death, infections being the leading cause [47].

The causes of renal failure in myeloma are multiple: light chain proteinuria, hypercalcaemia and dehydration, plasma cell infiltration, amyloidosis and hyperuricaemia. As mentioned before light-chain excretion is a very important factor to renal function decrease and indeed, patients without light-chain excretion seldom suffer from impaired renal function. The pathophysiology of light chain-induced renal damage has not been elucidated completely. Several mechanisms (e.g. tubular atrophy, intratubular cast forming) are probably responsible. The tubular atrophy is thought to be the result of a toxic action of light chains after they have been absorbed by the tubular cell [48].

The histologic picture of the so-called myeloma kidney shows dilated and atrophic tubuli many of which contain acidophilic casts

[49]. These casts consist of light chains, albumen, fibrinogen and non-monoclonal immunoglobulins [50]. Several authors suggested that aggregation and cast formation is the predominant pathway of light chain induced renal damage [51,52]. The aggregating and cast forming properties are probably dependent on intrinsic properties of the various light chains, such as the electrophoretic mobility [52,53].

Further abnormalities to be found are lympho-and/or plasmacytic infiltration, signs of infection and nephrocalcinosis [54,55]. Amyloid deposition is found in 8% of the patients [49]. The glomeruli are frequently normal, although EM-investigations disclosed endothelial and mesangial cell hyperplasia as well as thickening of the glomerular basement membrane [56].

Acute renal failure in myeloma has been reported in association with intravenous pyelography combined with dehydration. In total 26 cases were published [57]. This type of acute renal failure is probably caused by a precipitation of aggregates of light chains and the iodine preparation used for the radiographic study. In vitro research showed that light-chains (both kappa and lambda) precipitated when some iodine preparations were added to urine. In later prospective studies 609 intravenous pyelographic studies were done in 533 patients, resulting in acute renal insufficiency in 5 cases [57]. In properly hydrated patients intravenous pyelography is a safe procedure.

1.4 Diagnosis

In a patient presenting with pathological fractures, anaemia, a m-protein and a bone marrow aspirate with a marked increase of plasma cells the diagnosis of multiple myeloma is not difficult. In patients with only some of these symptoms, the diagnosis of multiple myeloma can be problematical. For example, does a patient with a low level m-protein and 10% plasma cells in an otherwise normal bone marrow deserve the diagnosis multiple myeloma? Frequently, the differentiation between MGUS and myeloma poses a problem. Furthermore, skeletal lesions in an old patient with a small increase in bone marrow plasma cells and a low serum m-protein level can just as well be caused by metastasis from a hitherto undetected malignancy, the m-protein being MGUS.

These diagnostic problems led to the development of several sets of diagnostic criteria. Two of these sets are shown in Table 3 [59,60]. Both have their disadvantages. For example, the criteria of the CALGB require more then 20% plasma cells in bone marrow aspirates, but this criterion cannot always be met because of sampling

error. It is recommended that, when suspicion is strong, several samples should be taken.

Table 3. Two sets of criteria for the diagnosis Multiple Myeloma.

A. I. Myeloma cells in excess of 20% on a count of 1,000 cells or sheet-like replacement by myeloma cells.

Plus

II. Abnormality of immunoglobulin production
A. Any of the following
1. Monoclonal spike greater than 4 g%
2. Rising monoclonal spike followed annually
3. Bence Jones protein in excess of 0.5 g/24 h.

Or (if the criteria for IIA are not satisfied then any combination of B and C)

B. Any of the following
1. Monoclonal spike less than 4 g% with reciprocal depression of normal immunoglobulins
2. (Pan) hypogammaglobulinemia

And

C. Any of the following
1. Osteolytic lesions when other causes have been excluded
2. Absence of other diseases characterized by marrow plasmacytosis: collagen disease, chronic infection, metastatic carcinoma, rheumatoid arthritis, viral exanthem; the presence of amyloid does not necessarily exclude the diagnosis of myeloma.

B. Major criteria.

I. Plasmocytosis on tissue biopsy
II. Bone marrow plasmocytosis of more than 30%
III. Monoclonal globulin spike on serum electrophoresis exceeding 3.5 g/100 ml for IgG peaks or 2.0 g/100 ml for IgA peaks; unequivocal evidence of kappa or lambda chain excretion on urine electrophoresis in the absence of amyloidosis.

The minor criteria are:

a. Bone marrow plasmocytosis of $\geqslant$ 10% but less than 30%.
b. Monoclonal globulin spike present but levels than the levels defined above.
c. Lytic bone lesions.
d. Normal IgM less than 50 mg/100 ml, IgA less than 100 mg/100 ml, or IgG less than 600 mg/100 ml.

Diagnosis is confirmed when any of the following criteria or combinations of criteria are documented in symptomatic patients: (1) I+b, I+c, or I+d; (2) II+b, II+c, or II+d; (3) III; (4) a+b+c or a+b+d. The presence of certain nonspecific disease features supports the diagnosis, including anaemia, hypercalcaemia, azotaemia, demineralization and compression fractures, and hypoalbuminaemia.

A: Cancer and Leukemia Group B, 1974 (ref. 59);
B: Southwest Oncology Group, 1982 (ref. 60).

It has to be stressed that diagnosing a patient as having multiple myeloma does not necessarily mean that treatment has to be started (see Disease Staging, paragraph 1.5). When the criteria of Table 3 are strictly applied, some of the patients diagnosed as having myeloma, will, on further follow-up, show to behave like a MGUS. These patients are then called 'smouldering myelomas' [61]. Smouldering myeloma is probably not really different from MGUS. Diagnostic aspects of solitary plasmocytoma already have been mentioned in paragraph 1.2.

1.5 Disease staging

There is obviously a great difference in patients presenting with multiple myeloma. On one side of the scale there is the patient without symptoms in whom suspicion is raised because of an elevated ESR during a routine check-up. On the other side is the patient suffering from severe skeletal pains, hypercalcaemia, anaemia, weight loss and with a very high m-protein level. These differences are – at least for a great deal – explained by different 'tumour-loads' or TBMC (Total Body Myeloma Cell number).

Beside the clinical symptoms, the TBMC is also reflected in the m-protein level. In 1970 Salmon and Smith determined the TBMC in ten patients with IgG myeloma. The basis for these determinations is shown by the formula:

$$\text{TBMC} = \frac{\text{total m-protein production (gram/day)}}{\text{m-protein synthesis per myeloma cell } (10^{-11} \text{ gram/day})}$$

Total m-protein production was calculated after determination of the plasma volume, the fractional catabolic rate of the different immunoglobulins and the serum m-protein level. The m-protein production per cell was measured in in vitro cultures of myeloma cells of the ten patients. The IgG production per cell ranged from 0.5-3.4 $\times$ 10^{-11} gram/day (12,500-85,000 molecules IgG/minute). In this small patient population no correlation existed between the IgG secretion rate and the degree of maturation as expressed by the morphology of the plasma cell [62].

In a later report TBMC data of 71 untreated patients were correlated with the presenting clinical features [63]. Bivariate correlation and multivariate regression analysis showed that the TBMC could be predicted on the basis of the presenting clinical features (extent of bone lesions, haemoglobin level, serum calcium level and m-protein level). Based on these data a staging system for myeloma patients was developed and published (Table 4).

Table 4. Staging system according to Durie and Salmon (1975, (63) left). Corresponding tumour cell mass, right. 10^{12} cells = approximately 1 kg, m^2 = square meter of body surface area.

Criteria	Measured myeloma cell mass (cells $\times 10^{12}/m^2$)
Stage I: All of the following:	
1. Haemoglobin value > 100 g/l	
2. Serum calcium value normal (≤ 3 mmol/l)	
3. On X-ray, normal bone structure (scale O) or solitary bone plasmacytoma only	
4. Low M-component production	< 0.6 (low)
(a) IgG level < 5 g/100 ml	
(b) IgA level < 3 g/100 ml	
(c) urine light chain M-component on electrophoresis < 4 g/24 h.	
Stage II: Fitting neither stage I nor III	0.6–1.20 (intermediate)
Stage III: One or more of the following:	
1. Haemoglobin value < 85 g/l	
2. Serum calcium value > 3 mmol/l	
3. Advanced lytic bone lesions (scale 3)	
4. High M-component production	> 1.20 (high)
(a) IgG level > 7 g/100 ml	
(b) IgA level > 5 g/100 ml	
(c) urine light chain M-component on electrophoresis > 12 g/24 h.	
Subclassification:	
A = relatively normal renal function (serum creatinine value < 180 μmol/l)	
B = abnormal renal function (serum creatinine value > 180 μmol/l)	
Examples:	
Stage IA = low cell mass with normal renal function	
Stage IIIB = high cell mass with abnormal renal function.	

This staging system gained international acceptance, and is currently the one most frequently employed. Staging the individual patient is done by obtaining the various parameters*. These can then be compared with the values in the staging system. This has to be done before chemotherapy is started. For stage I all criteria should be met, for stage II or III, only one criterion is sufficient. Assessing the renal function should be done after proper rehydration. When using this system, it has to be reminded that the abnormal parameters should be caused by the myeloma process. For instance, a haemoglobin level of

60 g/l due to severe haemorrhage or a haemolytic state does not automatically indicate stage III disease.

A staging system can be used for comparison of different therapeutic regimens in clinical trials or for selection of bad risk patients. The staging of an individual patient may also be of help in the decision whether therapy should be started. In general, patients with stage I disease or asymptomatic stage II disease should not be treated unless signs of progression are noted. The reason for witholding therapy in these patients initially, is the possibility that they may behave as smouldering myeloma (see paragraph 1.4). Frequent determinations

* With the regression equations (Table 5) on which the staging system is based, a more precise estimation of the TBMC (at presentation) can be calculated. Although this is useful for research purposes, clinical decision making is not influenced by the TBMC. With the aid of a programmable pocket calculator it is possible to perform the TBMC calculations very quickly. Another way to calculate TBMC in Bence Jones myeloma is given in reference 64, but this formula presumes the possibility to measure the light-chain production per plasma cell in the individual patient. After determining the TBMC at the time of diagnosis, changes in TBMC (for instance after the start of treatment) can be calculated. For IgA myelomas the katabolic rate of the m-protein is fixed, therefore the changes in m-protein reflect the changes in TBMC, provided the plasma volume does not change. When changes in plasma volume do occur the formula

$$M = (\frac{Cf \times Vf}{Ci \times Vi} - 1) \times 100 \text{ can be used.}$$

M is the change in TBMC in %, Cf and Ci are the m-protein levels (initial and follow-up) and Vf and Vi are the plasma volumes (initial and follow-up). For IgG myeloma the fractional catabolic rate for the m-protein depends on the m-protein level. The formula for calculating changes in TBMC in IgG myeloma is incorporated in the computer program in reference 65, in which the programs for the Hewlett-Packard-65 pocket calculator can be found.

Table 5. Equations for calculation of tumour mass at time of diagnosis.

A. Myeloma cell mass (cells $\times 10^{12}$)/m^2 = 0.413 + 0.256 × bone lesions + 0.019 × urine M component - 0.059 × haemoglobin + 0.065 × serum calcium + 0.050 × serum M component.

B. Myeloma cell mass (cells $\times 10^{12}$)/m^2 = 0.601 + 0.283 × bone lesions + 0.031 × urine M component - 0.058 × haemoglobin + 0.051 × serum calcium + 0.028 × serum M component

Bone lesions on skeletal X-ray survey are stated on a scale of 0 to 3: 0, normal bones, 1, osteoporosis only; 2, lytic bone lesions; 3, extensive skeletal destruction and major fractures. Values for laboratory tests are entered directly as haemoglobin (g/100 ml), serum M component (g/100 ml), serum calcium (mg/100 ml), and urine M component (g/24 hr).

A = especially suitable for IgG myeloma; B = can be used for IgG, IgA and Bence Jones myeloma.

of the m-protein level are indicated in order to establish the proper moment for the start of treatment.

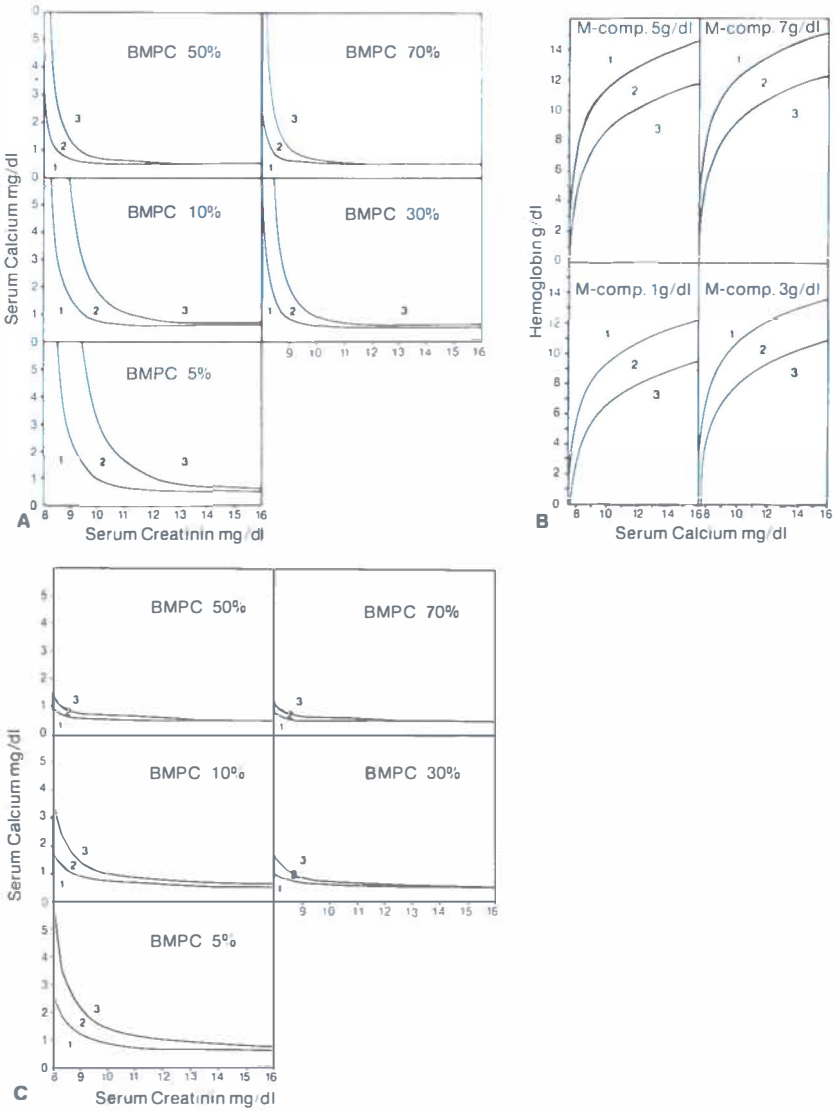

Fig. 1. A: graphs for staging in IgG myeloma; B: graphs for staging in IgA myeloma; C: graphs for staging in Bence Jones myeloma.
Since there is more than one graph for each group (IgG, IgA, BJ), the one with the value of the relevant variable (BPMC, M-component, BPMC respectively) closest to the observed value should be used.
BPMC = bone marrow plasma cell percentage.
1, 2 or 3 indicates the disease stage. For survival duration see text.

In 1980 Merlini, Waldenström and Jayakar published a different staging system [66]. This system is not based on the TBMC but on the multivariate regression analysis of presenting features in relation to measured survival. The patient population comprised 123 patients, observed between 1960 and 1971. Most patients had been treated with melphalan and prednisone. Analysis took place in 1978 when all but one patient had died. This staging system also distinghuishes three stages: stage-I patients live longer dan 50.1 months, stage-II patients live from 31.6 to 50.1 months and stage III-patients survive less than 31.6 months. Staging is simple using the nomograms from Fig. 1. These data should be obtained on admission of the patients before any treatment has been started. For patients with Bence Jones or IgG myeloma serum creatinine (mg/100 ml), serum calcium (mg/100 ml), and the percentage of plasma cells in a bone marrow aspirate are used. For IgA myeloma the IgA serum level (g/100 ml), serum calcium level (mg/100 ml) and haemoglobin level are needed. By using the regression formulas (Table 6) survival for an individual patient can be predicted. The predictive properties of this system will be discussed in paragraph 1.7.

For initial staging both systems can be used. The advantage of the D/S system is that it already has been used many times and by different investigators, in contrast to the Merlini system. Furthermore, the D/S system is based on TBMC while the Merlini system is based on a relation between survival and presenting symptoms in patients treated with melphalan/prednisone. It is therefore possible that the Merlini-system may not hold true for patients treated with other chemotherapy.

Table 6. Equations for calculation of survival for different myeloma classes (according to Merlini et al, 1980, ref. 66).

IgG myeloma survival (months)	$= 2.2768 - 0.6554 \times \text{calcium} - 0.3407 \times \text{BMP\%} - 0.2935 \times \text{creatinine}$.
IgA myeloma survival (months)	$= 1.2924 + 0.0727 \times \text{haemoglobin} - 0.4066 \times \text{calcium} - 0.3455 \times \text{M-component}$.
BJ myeloma survival (months)	$= 1.9408 - 0.5586 \times \text{creatinine} - 0.5045 \times \text{BMP\%} - 0.6223 \times \text{calcium}$.

calcium = serum calcium in mg/dl.
creatinine = serum creatinine in mg/dl.
BMP% = percentage of plasma cells differential count in a bone marrow aspirate.

The following transformations are required:

survival	$= \log x$
creatinine	$= \log (x - 0.5)$
calcium	$= \log (x - 7.5)$
BMP%	$= \log (x + 1)$.

1.6 Treatment

1.6.1 History

The first patients including Mr. McBean had been treated with venasection [67]. In 1901 laminectomy was performed on two patients, because of spinal cord compression [68]. Until 1946 radiation was the only available treatment having some effect in myeloma patients. In this year the first drugs for this disease were introduced.

Chemotherapy started with diamidine derivatives (stilbamidine and pentamidine), but therapeutic doses caused unacceptable toxicity [69]. In 1947 urethan (ethylcarbamate) was introduced and inital results were promising. However, a placebo-controlled trial showed a shorter median survival for the urethan-treated patients, and the use of urethan was consequently abandoned [70]. In 1955 melphalan (phenylalanine mustard) was reported to be effective in several animal tumours. These reports were followed by case reports which showed melphalan to be effective in multiple myeloma. In the 1960's therapeutic effects of melphalan were established beyond doubt.

The median survival time of patients before the introduction of chemotherapy has been investigated in several studies (Table 7). Median survival times of untreated patients ranged from 3.5-12 months.

Table 7. Median survival times of multiple myeloma patients not treated with chemotherapy.

Date	Ref. no.	No. of patients	Median survival from diagnosis in months
1960	71	238	3.5
1963	72	25	4
1966	70	15	12
1970	73	28	6

1.6.2 Chemotherapy 1960-1984

Before addressing the data concerning chemotherapy in multiple myeloma, it has to be stressed that between the published studies great variation exists in patient numbers, patient characteristics, response criteria and survival time calculation. This makes comparison of different studies difficult and sometimes impossible.

1.6.2.1 Single alkylating agent therapy

Since 1960 several studies have been published on myeloma treatment with melphalan or cyclophosphamide (both alkylating agents derived

from nitrogen mustard) with or without concomittant use of prednisone. Drug schedules varied from continuous low-dose administra-

Table 8. Single alkylating agent therapy in multiple myeloma.

Date	Ref. no.	Drug schedule[a]	No. of patients	Response rate % [b]	Median survival time fromstart of treatment, in months
1964	74	Cy 2 mg/kg/day	165	48	24.5
1968	75	Me 0.7–1.3 mg/kg over 4 days every 6 weeks	68	59	23
1969	76	Me 0.025 mg/kg/day continued	31	19	18
		Me 0.25 mg/kg/day for 4 days every 6 weeks	63	35	18
		Me 0.25 mg/kg/day for 4 days every 6 weeks, P 1 mg/kg on every monday, wednesday and friday	26	65	24
		Me 0.25 mg/kg/day + P 2 mg/kg/day for 4 days every 6 weeks	45	73	
1969	77	Me 0.1 mg/kg/day continued	54	15	15.5
		Cy 2–4 mg/kg/day continued	49	18	12.3
1970	78	Me 4 mg/day continued	39	41	28
1971	79	Me 4 mg/day continued	133	not given	18
		Cy 150 mg/day continued	141	not given	18
1972	80	Me 0.015–0.03 mg/kg/day continued	45	29	16.1
1977	81	Me 0.25 mg/kg/day + P 2 mg/kg/day for 4 days every 6 weeks	32	75	29
1979	96	Me 0.05 mg/kg/day continued (after induction dose of 0.15 mg/kg/day for 7 days) + P for 10 weeks	127	56	25
1979	82	Me 9–12 mg/m^2 + P 100 mg for 4 days every 4 weeks	120	72	28
1980	83	Cy 150 mg/day continued	124	not given	20
		Me 10 mg/day for 7 days every 6–8 weeks	128	not given	20
		Me 10 mg/day + P 40 mg/day for 7 days every 6–8 weeks	120	not given	20
1982	84	Me 8 mg/m^2 + P 75 mg for 4 days every 6 weeks	72	43	19
1983	85	Me 100-140 mg/m^2 i.v. once	5	100	–

a) Cy = cyclophosphamide; Me = melphalan; P = prednisone.
b) Response indicated by a decrease of M-protein level ⩾ 50%.

tion to high-dose pulse therapy. Results obtained with these different drug-schedules are shown in Table 8. From these data it seems clear that melphalan and cyclophosphamide are equally effective. Recently it was shown that intestinal absorption of melphalan ranged from 32-100% [86]. Therefore the optimal dose should be established in every individual patient.

Is the concomittant prescription of prednisone of benefit? The study by Alexanian et al. (1969, Table 8) showed an increase of response rate and survival time when prednisone was given with melphalan. In a study by Costa et al. (1973) this could only be shown for 'good-risk' patients, while 'poor-risk' patients showed a shorter survival time [87]. Many studies indicate an increased feeling of 'well-being' and a more rapid increase of haemoglobin level when prednisone is used. One study using only prednisone (200 mg every other day) without alkylating agents, showed a reduction of m-protein in eight of ten patients; haemoglobin level increased in six and skeletal pains decreased in five patients [88]. An in vitro study [89] showed that OAF-induced bone resorption (see paragraph 1.3) was blocked by cortisol (Raisz et al. 1975). Although a definite 'cytostatic' effect of corticosteroids on myeloma cells remains to be established, the above described data resulted in the general acceptance of corticosteroids for treatment of multiple myeloma.

Is the intermittent alkylating therapy to be preferred over continuous drug administration? As far as survival times are concerned this question cannot be answered with definite certainty, although several studies suggest a positive answer. Intermittent pulse therapy, when compared to continuous melphalan results in less frequent and prolonged bone marrow depression [81,87]. Furthermore, on theoretical grounds high-dose intermittent therapy may be expected to induce less resistance development than low-dose continuous treatment. Nowadays pulse therapy is therefore preferred and continuous therapy generally has been abandoned.

1.6.2.2 Multiple-drug regimens

In comparison with untreated patients administration of single alkylating agents resulted in a significant increase in median survival. However, most investigators were not satisfied with these results and this stimulated the search for more effective therapy regimens. In analogy to the MOPP-treatment for Hodgkin's disease multiple-drug regimens were investigated. A host of different combinations were tried and their effects are summarized in Table 9. Looking at these different regimens and their results several questions do arise: a) which regimen is the best? and is it really better than melphalan/prednisone? b) is the increased toxicity of these regimens acceptable

when compared with the increase in response rate and survival? These questions are not easily and with certainty answered because of different response criteria, differences in patient populations and because many studies were not randomized, but compared with historical data.

Table 9. Multiple-drug regimens in multiple myeloma.

Date	Ref. no.	regimen[a]	No. of patients	Response rate %	Median survival from start of treatment, in months
1972	91	MPrP	236	59[b]	23
1974	92	VMCBP	18	66[b]	not given
1977	93	MAP	76	46[b]	25
		MCP	85	47[b]	25
		MBCP	76	49[b]	34
		CAP	59	39[b]	25
		VCAP	48	57[b]	34
		VMCP	54	62[b]	34
1977	94	VMCBP	46	87[c]	40
1979	95	BCP	126	50[c]	27.8
1979	96	MBCP	124	68[c]	21 (poor-risk) 28 (good-risk)
1979	82	MBCP	83	60[b]	31
		MP	111	39[c]	24
1980	97	me-CCP	67	48[c]	32
1981	98	CAP	57	48[b]	32
		VCAP	87	67[b]	32
		VMCP	94	60[b]	30
		VBAP	51	65[b]	32
1982	105	BCP	70	50[c]	24.6
1982	99	VMCBP	25	81[c]	42
1984	100	MP	30	53[c]	38.4
		VMCP/VCAP alt.	42	55[c]	26.9
		VMCPx3/VBAPX3, alt.	34	60[c]	28

a) M = melphalan, Pr = procarbazine, P = prednisone, A = adriamycin, C = cyclophosphamide, B = BCNU, V = vincristine, me-C = methyl-CCNU;
b) response indicated by an M-protein decrease of more than 50%;
c) response indicated by an M-protein decrease of more than 75%.

The following is an attempt at interpretation of the available data. Treatment with a combination of melphalan, cyclophosphamide, BCNU and prednisone did not result in improved survival when compared with melphalan/prednisone (two randomized studies [82,96]. One study [96] showed that 'poor-risk' patients fared better

on the four-drug regimen and 'good-risk' patients fared worse when compared with melphalan/prednisone-treated patients. This observation, however, was not confirmed by Bergsagel et al. [82]. Regimens containing vincristine resulted in somewhat higher response rates and longer median survival times when compared with historical controls. In several regimens adriamycin did not result in an improved response rate or increased survival. Special attention deserves the so-called M-2 regimen [92,94,99]. This consisted of vincristine, melphalan, cyclophosphamide, BCNU and prednisone. In 1977 it achieved an 87% response rate (50% decrease in m-protein level) and a median survival of 40 months but this study has been criticized for inclusion of too many 'good-risk' patients. Furthermore, survival was calculated from 'diagnosis' and not from the start of treatment. In 1982 Tirelli et al. reported a response rate of 81% (75% decrease of m-protein level) with the M-2 regimen [99]. Median survival time was 42 months in this relatively small study (25 patients).

A recent study randomized patients for three different treatment modalities [100]: a) melphalan/prednisone, b) three courses of vincristine/melphalan/cyclophosphamide/prednisone (VMCP) alternated with three courses of vincristine/BCNU/adriamycin/prednisone (VBAP) and c) alternating courses of VMCP and vincristine/cyclophosphamide/adriamycin/prednisone (VCAP). The respective response rates were 53%, 60%, and 55% (response defined as a decrease of m-protein of more than 75%). Respective median survival times were 38.4, 28, and 26.9 months. The longer survival time for melphalan/prednisone was ascribed to the higher frequency of favourable prognostic factors in these patients.

What conclusions can be drawn from these studies? Although several studies support that multiple drug regimens result in higher response rates and longer median survival times than melphalan/prednisone, this has not been proved beyond doubt. This is important, especially since all multiple-drug regimens induce more toxicity then melphalan/prednisone. Additional studies are needed in which poor-risk patients (stage III) are randomized for melphalan/prednisone and multiple-drug regimens. At this moment melphalan/prednisone remains the therapy of choice for patients with symptomatic stage IA or IIA disease.

1.6.2.3 Maintenance therapy

In several studies an increased incidence of acute leukaemia was observed [82,101,102,104]. The rates, dependent on the duration of observation, varied from 10-19.6%. The incidence rises steeply after two years of treatment. However, exposure to alkylating agents is not the only cause for the increased leukaemia incidence, since patients

with other diseases treated with these agents do not show the same high incidence. Furthermore, 41 patients were described with myeloma and leukaemia who had not been treated [103]. Because of the leukaemogenic potential of alkylating agents long term treatment remains unattractive. The question therefore arose whether continued (maintenance) treatment was of any value regarding the survival duration.

In 1977 Alexanian et al. described 116 patients with a response (m-protein decrease more than 75%) to MVPP who were randomized for a) no maintenance therapy, b) melphalan/prednisone or c) BCNU/prednisone. The survival calculated from randomization did not differ for these three groups. The period from randomization to relapse was significantly longer (median nineteen months) for patients without a residual m-protein than for patients (median six months) with a residual peak [93]. Also, patients with a low TBMC at diagnosis had longer remission durations then patients with a high TBMC. In 80% of the relapsing patients a second remission (more than 50% reduction of m-protein) could be obtained with melphalan/prednisone.

In 1981 similar data were published on 69 responding patients who received no treatment after a six months induction therapy and twelve months therapy with VMCP and BCG. The median remission duration was nine months. Patients with a residual m-protein relapsed with a median of six months, and all had a relapse within twelve months [98]. For patients responding to the M2-protocol (Paccagnella et al., 1983) unmaintained remissions had a mean duration of 11.4 months for stage III disease and 24.6 months for stage I and II. After relapse the same treatment resulted in a remission (m-protein decrease more than 50%) in 82% of the patients [104].

In conclusion: patients without residual m-protein after initial therapy (which is arbitrarily continued for 12 months) will remain in remission for a limited period, but will ultimately relapse. A second tumour regression can usually be obtained when – at relapse – the initial form of therapy is restarted. Patients with a decrease in m-protein of 75%, – but with a residual peak – can also be followed without maitenance therapy but chances for a long term remission are smaller, especially if an advanced stage existed initially.

1.6.2.4 Treatment of patients with progressive disease during melphalan/prednisone therapy

The majority of patients shows some form of response to melphalan/-prednisone, ranging from a significant response (more than 75% decrease of TBMC) to a short-term stabilization of the m-protein level. Since cure of multiple myeloma is extremely rare, ultimately in

all patients the disease will relapse which is usually indicated by an increase of m-protein level and the occurrence of clinical symptoms. In addition, a small group of patients is completely unresponsive to melphalan/prednisone from the start of treatment (primary resistance). For these relapsing and resistant patients some other form of treatment must be chosen.

Several studies concerning 'rescue-therapy' were published. The results for single agents are shown in Table 10. It has been shown that primarily resistant patients have less chance of response to rescue therapy than relapsing patients [119]. Since initially all studies contained relapsing and resistant patients it is clear that the ratio of these two categories may be an important factor when evaluating the different studies.

Table 10. Single agent therapy in melphalan-resistant multiple myeloma.

Date	Ref. no.	Regimen	No. of patients	Response rate %[a]	Median survival in months
1962	105	mitomycine C	16	0	–
1963	106	vinblastine	17	0	–
1964	107	6-thioguanine	15	0	–
1970	108	procarbazine	28	14	–
1972	109	cyclophosphamide 1000 mg/m² i.v./ 3 wk, or 250 mg/m² p.o. for 4 days	19	31	21
1975	110	adriamycin	9	22	6
1976	111	Me-CCNU/prednisone	33	18[b]	–
		CCNU	10	0	–
1977	112	hexamethylmelamine	17	24	–
1978	113	adriamycin	13	12	–
		bleomycin	24	5	–
1979	114	pyrazofurin	14	7	–
1979	115	cyclophosphamide 600 mg/m²/ 3 wk iv. + prednisone 40 mg for 7 days	47	7	9.6
1980	116	vincristine 2 mg/week	4	50	–
1981	117	cyclophosphamide 325 mg/m² p.o. for 4 days	55	0[c]	–
1982	118	cyclophosphamide 150–300 mg/m² i.v. weekly + prednisone 50–100 mg every other day	5	60	–

a) response indicated by a decrease of the m-protein level of more than 50%;
b) response indicated by a decrease of the m-protein level of more than 75%;
c) this study contained 40 patients who were initially melphalan resistant.

Unfortunately several studies do not present separate numbers of the two categories, but only the results for the patients as a single group. What can be learned from the available data? It seems clear that mitomycine-C, vinblastine, 6-thioguanine, procarbazine, bleomycin, pyrazofurin, CCNU, and M-CCNU do not have therapeutic value for relapsing / resistant myeloma. Adriamycin (25-45 mg/ m^2 every 3 weeks) resulted in two partial responses in nine patients in one study [110] and one partial response in thirteen patients in another study ([113], adriamycin dose 60 mg/ m^2 every 3 weeks). The first study contained predominantly primary resistant patients, the second study consisted predominantly of relapsing patients. Adriamycin combined with BCNU resulted in three complete and four partial responses in thirteen patients [120], suggesting synergystic action of the two drugs (Table 11). This pilot study has not been followed by a study containing more patients. The response rate of 25% in the large VBAP study in which adriamycin and BCNU were also used (together with vincristine and prednisone) is disappointing [119].

Confusing are the data on single agent therapy with the alkylating agent cyclophosphamide (table 10). The first study [109] obtained a response rate of 31%, sharply conflicting with the response rates of 7% and 0% in the later studies [115,117]. Interesting is the observation that in the first study five of the six responders received the drug intravenously. This could explain why the third study (employing oral administration) produced such poor results. The poor results of the second study could probably be explained because the dose was lower (600 mg/ m^2) than in the first study (1000 mg/ m^2). The fourth study using cyclophosphamide every week (plus high dose prednisone) produced three partial responses in five patients [118]. Cyclophosphamide probably is effective when used in sufficiently high doses which are intravenously administered.

Of the vinca alkaloids, vinblastine was without any therapeutic effect but in a small study vincristine has been shown to induce tumour regression in patients with a partial response to alkylating therapy [116]. Only four relapsing patients have been treated with vincristine (Table 10). Three patients showed minor responses. In our unit vincristine has been used for treatment of relapsing patients (unpublished results). The administration of this drug had to be discontinued because of severe neurotoxicity. Vindesine, a new vinca alkaloid with less neurotoxic potential than vincristine was therefore tried in a phase II study (chapter 2). Vindesine showed anti-tumour activity, but responses were short-lived. This prompted us to combine adriamycin, cyclophosphamide, vindesine and prednisone in a rescue regimen. The experiences with this regimen are described in chapter 3.

Results of other multiple drug regimens in melphalan-resistant

Table 11. Multiple-drug regimens for treatment of melphalan-resistant multiple myeloma.

Date	Ref. no.	Regimen[a]	No. of patients	response rate[b] (%)	median survival in months
1976	120	AB	13	54	–
1977	94	VMCBP	26	50	22+
1978	121	ABCP	14	36	7
1979	115	BCP	42	17	9.5
1979	95	BCP	33	28	–
1982	119	VBAP	150	25	7
1982	122	CiBCP	23	22	–
1984	123	VAD	29	59[c]	–

a) A =adriamycin, B = BCNU, C = cyclophosphamide, P = prednisone, V = vincristin, Ci = cisplatin, D = dexamethasone;
b) Response indicated by a decrease of serum m-protein level of > 50%;
c) Response indicated by a decrease of serum m-protein level of > 75%.

myeloma are shown in Table 11. The highest response rates have been obtained with the M-2 regimen [94] and with a combination of vincristine, adriamycin (both administered in 24-hour infusion) and dexamethasone [124], but the numbers of patients were relatively small and additional studies are required. The largest study [119] showed a response rate of 25% with VBAP. The median survival was seven months for the whole population and twelve months for the patients with a response to VBAP. A relaps during treatment seems indicative of a poor prognosis.

1.6.3 Therapy of specific problems

1.6.3.1 Skeletal manifestations

a) Radiation therapy.

Indications for radiation therapy in myeloma are imminent spinal compression, pathological fractures, prevention of fractures (e.g. in the long bones), skeletal pain (ribs, vertebrae) and soft tissue localizations. Radiation doses vary according to the indication. For palliation of skeletal pain 15-25 Gy is sufficient in most cases. For pathological fractures and prevention of fractures a dose of 30-35 Gy seems adequate. For therapy of solitary myeloma or extramedullary plasmocytoma a dose of at least 50 Gy has been advocated since the goal is definite cure in stead of palliation [124]. Spinal compression results most frequently from extraosseous-growing plasmacytoma. Vertebral fractures may lead to compression. In early stages paralysis can be prevented by high-dose steroids in combination with radiation.

For patients showing already signs of paralysis emergency laminectomy is indicated [125].

A special form of radiation therapy is the total body radiation for patients who are resistant to chemotherapy. This form of therapy is still experimental [126].

b) Orthopaedic therapy

Surgical therapy is sometimes indicated for joint-replacement or stabilization of long-bone fractures. A frequent problem is the lack of supportive bone for the prosthetic material [127]. Conservative treatment with a spinal-supportive brace sometimes results in effective palliation of vertebral pain.

c) Medical treatment

Treatment with vitamin D (50,000 U twice/week), sodium fluoride (50 mg twice/day) and calcium carbonate (1 g four times/day) resulted in an increase in bone mass compared to controls [128].

A recent placebo-controlled trial reported no skeletal improvement from the use of vitamin D (50,000 U twice/week), sodium fluoride (150 mg/day), calcium gluconate (6 g/day) and fluoxymesterone (25 mg/m^2/day, [129]).

It must be born in mind that patients, responding to chemotherapy, are generally without symptoms and it is unlikely that, in the case of a disease relapse, this mode of treatment will prevent new bone lesions. Furthermore, the risk of hypercalcaemia may be increased by this medication. Treatment with diphosphonates, which presumably results in inhibition of the OAF-induced osteolysis, is still experimental.

1.6.3.2 Renal failure

Renal failure requiring dialysis treatment at the time of diagnosis of myeloma was reversible in only eleven of 63 patients. Long term dialysis in 44 patients was not associated with more problems than dialysis in non-myeloma patients [130,131]. These studies indicate that newly diagnosed myeloma patients with renal failure may benefit from long term dialysis, especially if chemotherapy is effective. Case reports suggest a beneficial effect of plasmapheresis and high-dose steroid pulses for these patients [132,133], by improving renal function to a level for which dialysis is not indicated. Dialysis is clearly not indicated for patients with non-responding or relapsing myeloma. Renal failure not requiring dialysis treatment, occurs frequently (see paragraph 1.3.3).

Treatment of contributing factors as hypercalcaemia, hyperuricaemia and institution of effective chemotherapy (resulting in decreased light chain production) is reported to result in a partial

reversibility of the renal function impairment in 55% of patients [134]. The survival time of these patients was increased when compared to those without reversibility. Finally, renal transplantation can be considered for patients in complete remission [131].

1.7 Prediction of prognosis in multiple myeloma

As in other diseases many investigators have been intrigued by the question whether it is possible to predict the prognosis of the individual patient at the moment of diagnosis. The general approach to this problem has been the application of a multivariant analysis on the relation between survival and presenting features.

1.7.1 Prognostic value of single clinical and laboratory data

There is general agreement that a decreased renal function (serum creatinine level above 180 μmol/l) or anaemia (haemoglobin level below 90 g/l) correlates with a significantly reduced survival [95,135-137]. Further agreement exists on the shorter survival for Bence Jones myelomas (especially the lambda-subclass) when compared with IgG and IgA myelomas [76,139]. This is partly explained by the frequently decreased renal function in light chain myelomas, while several authors suggest that light-chain myelomas have more malignant growth characteristics. The difference in survival between the kappa and lambda light chain myeloma patients could not be explained. This difference is also found within the group of IgG myelomas with light chain proteinuria: patients with kappa light chain proteinuria showed a median survival time of 31 months in contrast to the twelve months for the patients with lambda light chain proteinuria [140].

Hypercalcaemia, skeletal lesions and a low serum albumen level also indicated shorter survival but the predictive value of these factors is not significant when corrected for correlations with anaemia and renal function loss.

In recent years the relation between the serum β2-microglobulin (β2-m) level at presentation and survival of myeloma patients has been investigated. In a group of 37 patients the median survival time for patients with an initial β2-m < 4 μg/ml was 46 months, in contrast with 15 months for those patients with an initial β2-m > 4 μg/ml [141]. Bataille et al. produced similar data for a group of 160 patients [142]. In both studies serum β2-m was not corrected for renal function (serum creatinine). Since β2-m is rapidly excreted through the glomeruli, the serum β2-m level is very dependent on the status of renal function. The relation between renal function and serum β2-m

was investigated by Cassuto who found the following relation (in patients without lymphoid malignancies):

$\log_e \beta 2\text{-m} = 3.84 - 5.96\ Y + 2.94\ Y^2 - 0.476\ Y^3 + 0.0252\ Y^4$,
where $Y = \log_e$ serum creatinine; $r = 0.9849$ [143].

We investigated the predictive value of β2-m (corrected for serum creatinine levels) as an independent parameter for median survival in 87 myeloma patients. The results are given in chapter 4.

Several studies were done on the relation between survival and plasma cell morphology. Although many forms of plasma cells can be found, for practical purposes multiple myeloma can be divided in three morphological types: a) differentiated myeloma mainly consisting of mature plasma cells, b) intermediate myeloma consisting of a mixture of plasma cells and immature plasmablasts and c) undifferentiated myeloma consisting mainly of immature plasmablasts. In a study by Wutke et al. of 202 patients the median survival times for the respective morphologic types were: 39.7, 16.1 and 9.8 months [144]. In another study using the same criteria respective median survival times of 30.5, 16.4 and 4.6 months were found [145]. This study showed that patients with a high TBMC (greater than 2.0×10^{12} cells) more frequently had undifferentiated myeloma. The decreased survival time of patients with immature plasma cells and also the correlation between the degree of plasma cell infiltration of the bone marrow and the plasma cell immaturity, had already been reported in 1956 by Mandema [146].

Although multiple myeloma can be distributed unevenly through the skeleton, Vercellli et al. found a significant negative correlation between bone marrow infiltration (percentage of plasma cells in a marrow aspirate) and the median survival time [147]. This was also found by Merlini et al. [66]. Bartl et al. looked at the type of proliferation of plasma cells in iliac crest biopsies [148]. Three types of proliferation could be observed: a) diffuse infiltration, but the plasma cells are predominantly localised around vessels and along the endosteal surface, b) as a), but with the presence of plasma cells nodules, c) diffuse infiltration with bone marrow replacement. These different types of proliferation correlated with median survival times and also with plasma cell morphology. Mature plasma cells were found more frequently in type a) and immature plasmablasts occurred predominantly in type c). There was however a considerable overlap.

1.7.2 Prognostic value of disease stage

Does the staging system of Durie and Salmon have prognostic value? As may be expected, patients with the B-subclassification (indicative

of impaired renal function) have a shorter median survival than 'A'-patients.

In the publication of Durie and Salmon (1975) in which their staging system originally was described [63], a significant survival difference between the three stages (see paragraph 1.5) was reported (total 71 patients). In 1980 the same authors published the results on 150 patients as shown in Fig. 2 [149], again with significant survival differences between stages. Prior to this publication, Woodruff et al. (1979) had analysed the survival of 237 patients retrospectively and

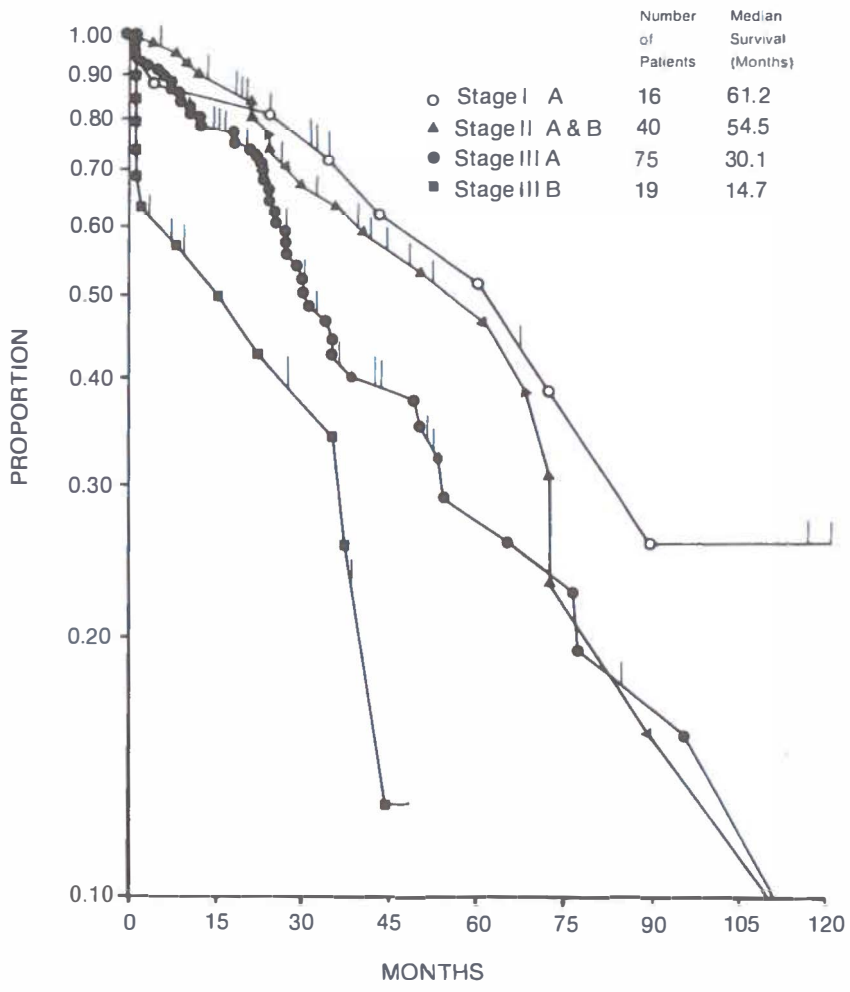

Fig. 2. Survival of 150 myeloma patients after stratification for disease stage (From: Durie BGM, Salmon SE, Moon TE. Blood 1980, 55, 364–372 with kind permission of the publisher).

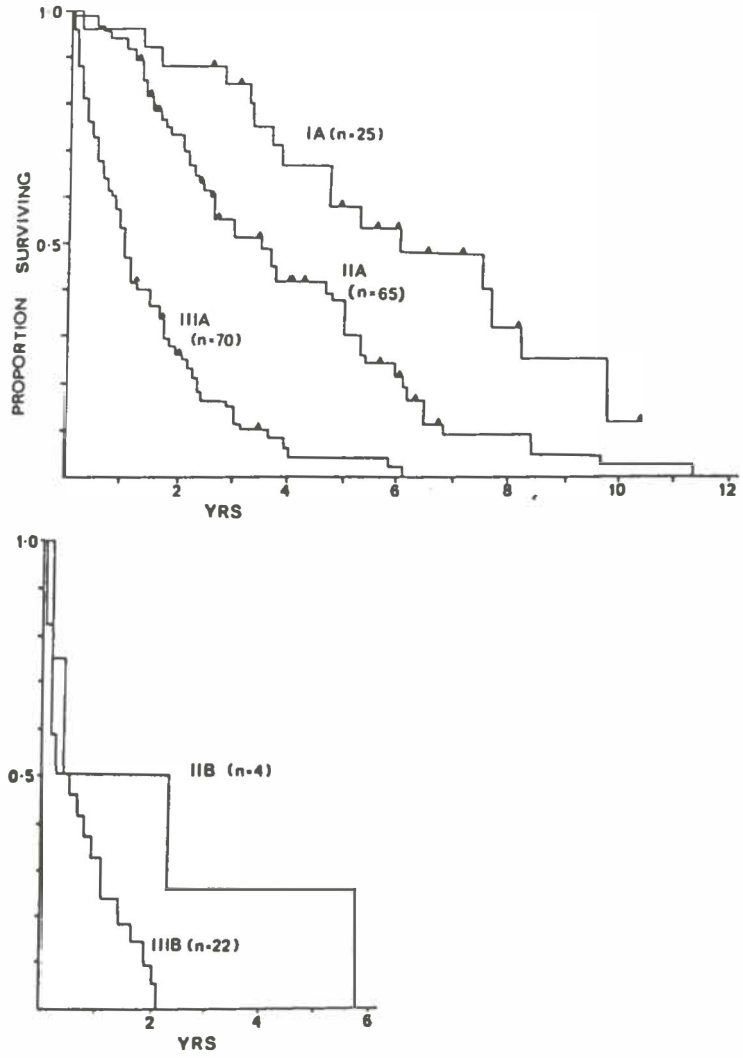

Fig. 3. Survival data of 185 myeloma patients; stratified according to Durie/Salmon (from: Woodruff et al, Brit. J. Haematol. 1979, 42, 199–205; with kind permission of the publisher).

Median survival

IA	72 months	IA vs IIA	$P < 0.0001$
IIA	37 months	IIA vs IIIA	$P < 0.0001$
IIIA	12 months	IA vs IIIA	$P \ll 0.0001$
II/IIIB	3 months		

had found highly significant differences in median survival times (Fig. 3) [150]. However, in a report of the findings in 98 patients, Jansen et al. did not find significant differences in survival [135].

The patients from the β2-m study (chapter 4) were also staged with the D/S system. Their survival data are shown in Fig. 4. Significant differences were found between stage IA and Stage IIIA, as well as between stage IIIA and Stage 'B'. We agree with most authors that the median survival of patients with stage III disease is significantly shorter compared with stage I/II disease. The CES-regimen which we designed (see paragraph 1.8 and Chapter 5) was for this reason administered to patients with stage III myeloma only. In our opinion patients with stage I or II should be treated with melphalan/prednisone.

Predicting survival for an individual patient remains a hazardous

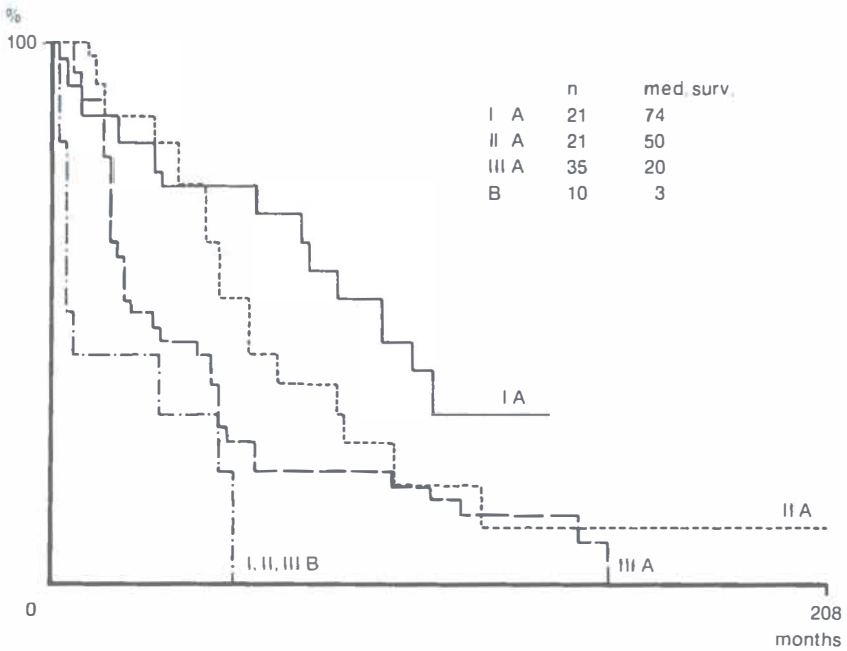

Fig. 4. Survival of 87 myeloma patients, stratified for their disease stage (Durie/Salmon).

	32	100	208 months
IA vs IIA	n.s.	$p < 0.05$	$p < 0.10$
IIA vs IIIA	$p < 0.05$	$p < 0.2$	$p < 0.2$
IA vs IIIA	$p = 0.025$	$p < 0.01$	$p < 0.025$
IIIA vs B	$p < 0.005$	$p \ll 0.0005$	$p \ll 0.0005$

practice since many studies show short-survivors in stage I disease and long-survivors in stage III disease. The staging system of Merlini et al. (see paragraph 1.5) supplies the possibility of predicting survival for an individual patient, and in their own study (in retrospection) survival was correctly predicted in 85% of the patients [66]. As emphasized earlier, this study was based on melphalan/prednisone treated patients, so the formulas for survival calculation may not hold true for other drug-regimens.

1.7.3 Prognostic effect of response to therapy

In the survival prediction of Merlini et al. the relation between response-to-therapy and survival duration is not mentioned. It is obvious that this is not an immaterial issue, but surprisingly few authors discuss this subject. In 1972 Alexanian et al. reported a median survival time of approximately sixteen months for non-responding patients versus 34 months for responding patients [91]. In a later study they described again a difference in survival time between responders and non-responders [151]. In this study it was shown that the chance of response was not dependent on the initial tumour load (disease stage). It was also shown that a responding patient with an initial high tumour-load could survive longer than a non-responding patient who presented with a low tumour load. In 1980 Cavagnaro et al. showed that responders survived significantly longer than non-responders [97].

Unfortunately no large scale studies have analysed this issue, but it appears that non-responders or primary resistant patients have a poor prognosis, and should probably be considered for more aggressive forms of treatment.

Several authors have reported on the finding that patients who respond very rapidly (reaching a complete or partial response within two or three months) survive shorter than patients who respond more slowly [152,153]. Subsequently it was found [154] that these patients were characterized by a large tumour mass with a high labelling index (LI), i.e. a high number of actively proliferating cells (see also paragraph 1.8). This correlates with results of another study in which 79 untreated myeloma patients were investigated [149]. TBMC (calculated on the basis of m-protein production per plasma cell, which was determined for every patient) and LI as well as the product (TBMC $\times$ LI) were compared with survival. TBMC (stage I etc) and LI both showed significant negative correlation with survival. TBMC $\times$ LI ($=$S, number of DNA synthesizing cells) correlated highly significant with survival: median survival time for $S < 10^{11}$ cells was 36 months, for $S > 10^{11}$ only 10 months (Fig. 5).

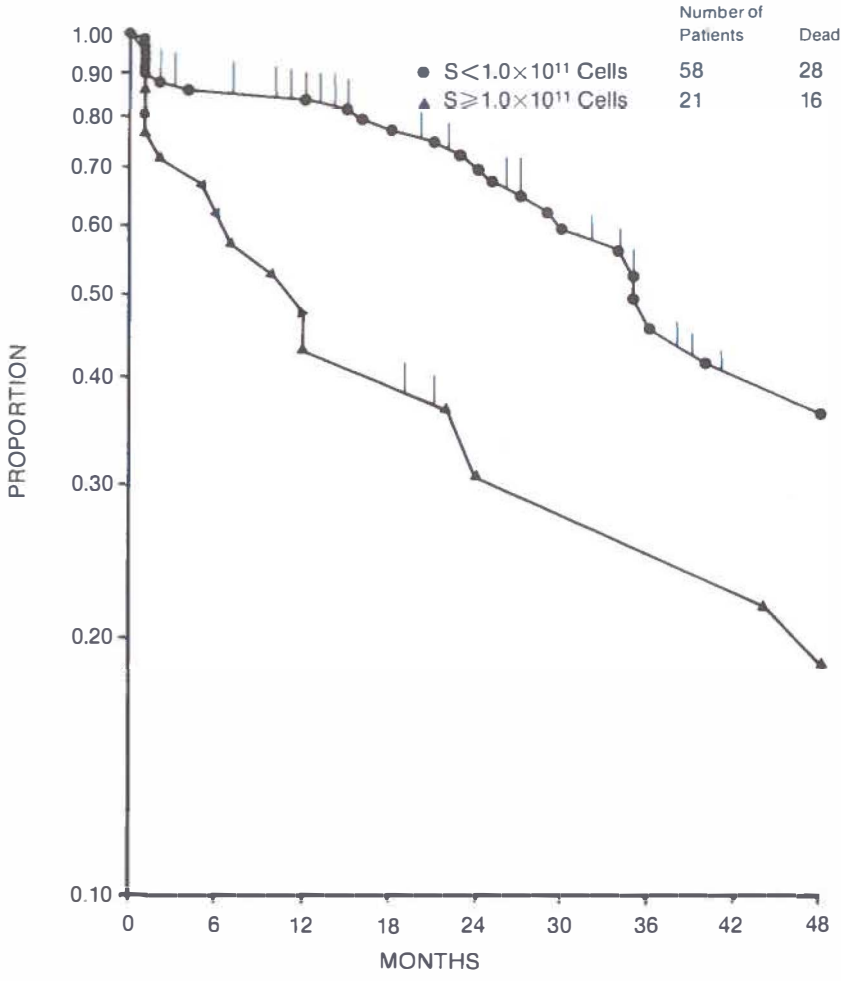

Fig. 5. Survival curves of 79 patients stratified for the number of DNA synthesizing cells S, (S = TBMC × LI).
Median survival for $S < 10^{11}$ cells is 36 months and for $S > 10^{11}$ cells 10 months ($p = 0.004$).

1.7.4 Prediction of response to therapy

In the study of Durie et al. [149] response to therapy could not be predicted, and separate survival times for responding versus non-responding patients were not given. A major factor regarding response and survival is the intrinsic drug-sensitivity of the malignant plasma cell.

Using cultured myeloma cells from patients it is possible to test tumour-drug-sensitivity in vitro (clonogenic assay); this sensitivity should then be compared to the clinical response. In sixteen previously untreated patients in-vitro sensitivity corresponded with response in twelve (75%) patients [154]. In 24 patients Ludwig [155] found the clinical response correctly predicted by in-vitro testing in 21 (87.5%) patients. Additional studies with larger patient populations are expected in the near future. Potential benefits of these in-vitro procedures may lie in individual therapy selection e.g. by witholding ineffective drugs, and in the area of new drug testing.

In vivo responses can be assessed by monitoring cell death. The polyamines putrescine, spermine and spermidine are closely related to cell proliferation and other cellular functions as e.g. protein synthesis. When cells die, these polyamines are released and elevated levels can be measured in biological fluids. Elevation of spermidine levels has been shown to correlate with cell death in animal systems as well as in human tumours [156,157]. We have therefore investigated plasma spermidine levels measured by radioimmunassay for their value as predictors of response to therapy (Chapter 6). This radioimmunoassay was developed by Jurjens et al. at the Laboratory for Nuclear Medicine of the University Hospital in Groningen.

1.8 Cytokinetics

Multiple myeloma is a disease with two outstanding features: a measurable specific tumour marker (m-protein) in the majority of cases, and the possibility to obtain tumour cells repeatedly, by sternal or iliac crest bone marrow aspiration. These properties have resulted in a large number of studies concerned with cytokinetic aspects. It was hoped, that the obtained information regarding the cytokinetic behaviour of the malignant plasma cells would contribute to the development of a more effective treatment. The issue has proven to be a difficult one, because the plasma cell population is not of a single cell type.

According to Drewinko et al. [158] a given myeloma cell mass probably consists of three different populations: a) plasma cells without capacity for cell division, b) plasma cells with limited mitotic capacity, and c) cells with stemcell capacity, i.e. the capacity for self renewal (like the stem cells of normal bone marrow).

In the description of cytokinetic behaviour of tumours three different parameters are used. 1: the clonogenic fraction (CF) is the percentage of tumour stem cells with the capacity for selfrenewal. With currently available techniques, this CF cannot be measured exactly. After plating a given number of tumour cells in an in vitro culture

medium (clonogenic assay) the number of clones provides only an approximate estimate of the CF. 2: the labelling index (LI) reflects the percentage of tumour cells which take up radioactive labelled thymidine during a certain exposure time. This LI (the number of cells in S-phase) theoretically depends on cells from the clonogenic fraction but possibly also of more mature – still dividing – plasma cells. 3: the growth fraction (GF) which is measured by the so-called H^3-thymidine suicide index, is that part (in %) of the CF which is actively engaged in cell division.

Whether cells in the clonogenic fraction are morphologically and/or functionally different from the more mature plasma cells is still a matter of debate. Bast et al. suggested that colony formation in the clonogenic assay could result from cells at the stage of plasma cells [159]. It must be understood that the magnitude of the LI does not necessarily correlate with the size of the GF. Although a small GF is always associated with a low LI, a low LI does not exclude the existence of a large GF [160]. This results from the fact that the GF is only a percentage and not an absolute number of actively proliferating stem cells. A high LI correlates positively with plating efficiency in the clonogenic assay [161]. In general, the LI has been thought to be a more or less reliable indicator of the proliferating activity of the tumour mass.

At the moment of diagnosis, before the start of therapy, about 60% of the patients has a low LI [153]. In some of these patients a large GF is found which probably indicates that a small number of actively proliferating stem cells is responsible for the slow increase in the TBMC. After the start of treatment most patients show a temporary increase in LI (possibly due to recruitment?), but the LI returns to a low level after significant tumour reduction and a stable plateau phase are reached [162]. These patients again show a high GF, again indicating a small population of cycling stem cells. At this moment administration of cycle phase-specific drugs could probably result in further tumour reduction. This approach was succesfull with vincristine but not with cytosine arabinoside, hydroxyurea or azathioprine [163]. Another approach could be witholding of all therapy, especially in patients with a tumour reduction of more than 75%.

In patients with a relapse the LI is frequently elevated as well as the GF [164]. In this patient group treatment with cell cycle specific agents (vinca alkaloids) could be beneficial. We therefore used vindesine in a phase-II study in melphalan-resistant myeloma patients (Chapter 2).

A subgroup of patients has a LI greater than 3% at the time of diagnosis. When this is accompanied with a large TBMC, treatment can result in an initial rapid decrease of TBMC without reaching a

plateau phase [149,153]. The LI of these patients increases and remains high which results in a rapid relapse. Initial therapy in these patients should probably be conducted with cell-cycle specific agents.

In 1977 and in 1981 Karp et al. published two papers in which they described a humoral stimulatory activity (HSA) in serum of myeloma patients which occurred six to eighteen days after cyclophosphamide administration [165,166]. Plasma cells stimulated with this HSA were more sensitive to the cytotoxic effects of adriamycin then plasma cells which were cultured in a normal environment. It should be investigated whether the increased LI after therapy is the result of such a HSA.

In Chapter 5 we describe our experiences with a cytotoxic regimen based on cytokinetic data.

1.9 Purpose of the study

Chapter 2:
Melphalan-resistant multiple myeloma patients were treated with vindesine and prednisone in order to evaluate whether vindesine had tumouricidal potential.

Chapter 3:
A multiple-drug regimen consisting of cyclophosphamide, adriamycin, vindesine and prednisone was evaluated for therapeutic and toxic effects in melphalan-resistant multiple myeloma patients.

Chapter 4:
In myeloma patients β2-microglobulin serum levels at the moment of diagnosis were investigated for their prospective value in terms of survival, as well as for their correlation with certain presenting features. The study was undertaken because of scarce and conflicting results in the literature.

Chapter 5:
Multiple myeloma patients with a high tumour mass have a shorter median survival time than patients with a low tumour mass when treated with melphalan/prednisone. On the basis of certain cytokinetic data a regimen was designed consisting of cyclophosphamide, high-dose methylprednisolone and vindesine. The effects of this regimen were evaluated in patients with a high tumour mass.

Chapter 6:
Survival of myeloma patients is for a part dependend on the response to treatment of the plasma cell mass. In vitro drug-sensitivity testing is

difficult due to technical problems. Polyamine (spermidine) levels reflecting cell death were measured in plasma, in order to evaluate their usefullness for drug-sensitivity testing in vivo.

References

1. Bence Jones H. Papers on chemical pathology' prefaced by the Gulstonian lectures read at the Royal College of Physicians, 1846. Proc Royal Soc London. Lancet 1849, ii, 88.
2. Bence Jones H. On a new substance occurring in the urine of a patient with 'mollities osseum'. Phil Tr Royal Soc London 1848, 55.
3. von Rustizky J. Multiples Myelom. Z f Chir 1873, 3, 102.
4. Kühne W. Hemialbumose in the urine. Z Biol 1883, 19, 209.
5. Kahler O. On the symptomatology of multiple myeloma. Observations of albumosuria. Prager Med Wschr 1889, 14, 33-45.
6. Wright JH. A case of multiple myeloma. Trans Ass Amer Physicians 1900, 15, 137.
7. Arinkin MJ. The intravital technique of the bone marrow examination. Folia Haemat Lpz 1929, 38, 323.
8. Kyle RA. In: Salmon SE (ed): Clinics in Haematology, W.B. Saunders Company Ltd, 1982, vol 11, no 1, page 124.
9. Houwen B. Diagnostiek en behandeling van multipel myeloom. In: Medisch Jaarboek 1980, Bohn, Scheltema en Holkema, Utrecht, pp 90-100.
10. Kyle RA. Monoclonal gammopathy of undetermined significance: natural history in 241 cases. Am J Med 1978, 64, 814-826.
11. Paladine G, Sala PG, Santini PA. Benign Bence Jones Gammopathy. Acta Haemat 1980, 63, 241-246.
12. Pruzanski W, Gidon MS, Roy A. Suppression of polyclonal immunoglobulins in multiple myeloma: relationship to staging and other manifestations at diagnosis. Clin Imm and Immunopathol 1980, 17, 280-286.
13. Peltonen S, Wasastjerna C, Wagner O. Clinical features of patients with a serum m-component. Acta Med Scand 1978, 203, 257-263.
14. Alexanian R. Monoclonal gammopathy in lymphoma. Arch Int Med 1975, 135, 62-66.
15. Zawadzki O, Benedek TG. Rheumatoid arthritis, dysproteinemic arthropathy and paraproteinemia. Arthritis Rheum 1969, 12, 555-568.
16. Michaux JL, Heremans JF. Thirty cases of monoclonal immunoglobulin disorders other than myeloma or macroglobulinemia: A classification of diseases associated with the production of monoclonal-type immunoglobulins. Am J Med 1969, 46, 562-579.
17. Shoenfeld Y, Berliner S, Pinkhas J, Beutler E. The association of Gaucher's disease and dysproteinemias. Acta Haemat(Basle) 1980, 64, 241-243.
18. Bataille R, Sany J. Solitary myeloma: clinical and prognostic features. A review of 114 cases. Cancer 1981, 48, 845-851.
19. Alexanian R. Localized and indolent myeloma. Blood 1980, 56, 521-525.
20. Wiltshaw E. The natural history of extramedullary plasmacytoma and its relation to solitary myeloma of bone and myelomatosis. Medicine 1976, 55, 217-238.
21. Bataille R. Localized plasmacytomas. In: SE. Salmon (ed): Clinics in Haematology, WB Saunders Company Ltd, vol 11, no 1, 113-122, 1982.
22. Fagiolo E, Tosato G. IgM plasmacytoma: report of a case and review of the literature. Haematologia 1978, 12, 221-229.

23. Bardwick PA, Zvaifler NJ, Gill GN, Newman D, Greenway GD, Resnick DL. Plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, M-protein and skin changes. The POEMS syndrome. Report of two cases and review of the literature. Medicine 1980, 59, 311-322.
24. Driedger H, Pruzanski W. Plasma cell neoplasia with peripheral polyneuropathy. A study of five cases and a review of the literature. Medicine 1980, 59, 301-310.
25. Kyle RA, Greipp PR, Banks PM. The diverse picture of gamma heavy-chain disease. Report of seven cases and review of the literature. Mayo Clin Proc 1981, 56, 439-451.
26. Kyle RA. Amyloidosis. In Salmon SE (ed): Clinics in Haematology, WB Saunders Company Ltd, vol 11, no 1, 151-180.
27. Horton JE, Raisz LG, Simons HA, Oppenheim JJ, Morgenhagen SE. Bone resorbing activity in supernatant fluid from human cultured peripheral blood leukocytes. Science 1972, 177, 793-795.
28. Mundy GR, Raisz LG, Cooper RA, Schechter GP, Salmon SE. Evidence for the secretion of an osteoclast stimulating factor in myeloma. N Engl J Med 1974, 291, 1041-1046.
29. Durie BGM, Salmon SE, Mundy GR. Relation of osteoclast activating factor production to extent of bone disease in multiple myeloma. Brit J Haematol 1981, 47, 21-30.
30. Ingeberg S, Deding A, Krogh Jensen M. Bone mineral content in myelomatosis. Acta Med Scand 1982, 211, 19-21.
31. Schechter GP, Wahl LM, Horton JE. In vitro bone resorption by human myeloma cells. In: Progress in myeloma (Potter M,ed). Elzevier/North Holland, New York, Oxford, Amsterdam. 1980.
32. Yoneda T, Mundy GR. Prostaglandins are necessary for osteoclast activating factor production by activated peripheral blood leukocytes. J Exp Med 1979, 149, 279-283.
33. Joss E, Burnstein SJ, Calabro JJ, Henderson ES. Multiple myeloma complicating the course of seronegative lupus erythematosus. Arthritis Rheum 1978, 21, 260-263.
34. Tamir R, Glanz I, Lubin E, Vana D, Pick AJ. Comparison of the sensitivity of 99-mTc-methyl diphosphonate bone scan with the skeletal X-ray survey in multiple myeloma. Acta Haemat 1983, 69, 236-242.
35. Ludwig H, Kumpan W, Sinzinger H. Radiography and bone scintigraphy in multiple myeloma: A comparative analysis. Brit J Radiol 1982, 55, 173-181.
36. Hobbs JR. Immunochemical classes of myelomatosis. Including data from a therapeutic trial conducted by a Medical Research Council Working Party. Brit J Haematol 1969, 16, 607-617.
37. Dreicer R, Alexanian R. Nonsecreting multiple myeloma. Am J Haematol 1982, 13, 313-318.
38. Ferraris AM, Haupt E, Ratti M. Multiple myeloma without detectable Ig synthesis. Acta Haematol 1979, 62, 257-261.
39. Osserman EF, Takatsuki K. Plasma cell myeloma: gammaglobulin synthesis and structure. Medicine 1963, 42, 357-361.
40. Brackenridge CJ, Lynch WJ. Some observations on myeloma and macroglobulinaemia. Med J Austr 1967, 54, 493-502.
41. Penny R. Paraprotein patterns in Australia. Austr Ann Med 1969, 18, 502-508.
42. Marrink J, Weltevreden E, Houwen B. Distribution of m-proteins in myeloma patients. University Hospital Groningen, The Netherlands, 1979. Unpublished results.

43. Block KJ, Maki DG. Hyperviscosity syndromes associated with immunoglobulin abnormalities. Semin Hematol 1973, 10, 113-124.
44. Preston FE, Cooke KB, Foster ME, Winfield DA, Lee D. Myelomatosis and the hyperviscosity syndrome. Brit J Haematol 1978, 38, 517-530.
45. Bronet JC, Clauvel JP, Danon F, Klein M, Seligman M. Biological and clinical significance of cryoglobulins. A report of 86 cases. Am J Med 1974, 57, 775-788.
46. Ludwig H. Multiples Myelom. Diagnose, Klinik und Therapie. Springer Verlag Berlin, 1982, page 102.
47. Kyle RA. Multiple myeloma: review of 869 cases. Mayo Clin Proc 1975, 50, 29-40.
48. De Fronzo RA, Cooke CR, Wright JR, Humphrey RL. Renal function in patients with multiple myeloma. Medicine 1978, 57, 151-166.
49. Schubert GE, Veigel J, Lennert K. Structure and function of the kidneys of patients with multiple myeloma. Virchow's Arch Pathol Anat 1972, 355, 135-157.
50. Levi DF, Williams RC, Linstrom FD. Immunofluorescent studies of the myeloma kidney with special reference to light chain disease. Am J Med 1968, 44, 922-933.
51. Clyne DH, Pollak VE. Renal handling and pathofysiology of Bence Jones protein. Contr Nephrol 1981, 24, 78-78.
52. Hill GS, Morel-Maroger L, Mery JP, Mignon F. Correlations between relative electrophoretic mobilities of light chains and renal lesions in multiple myeloma. Proc Amer Soc Nephrol 1978, 78 (abstract).
53. Fine JD, Luke RG, Rees ED. Multiple myeloma and renal involvement. Lancet 1973, ii, 1205-1206.
54. Pasmantier MW, Azar HA. Extraskeletal spread in multiple plasma cell myeloma: a review of 57 autopsied cases. Cancer 1969, 23, 167-174.
55. Kapadia SB. Multiple myeloma: A clinicopathologic study of 62 consecutively autopsied cases. Medicine 1980, 59, 380-392.
56. Fisher ER, Perez-Stable E, Zawadzki ZA. Ultrastructural renal changes in multiple myeloma with comments relative to the mechanism of proteinuria. Lab Invest 1964, 13, 1516-1574.
57. Gassmann W, Haferlack T, Schmitz N, Kayser W, Löffler H. Zur Problematik des intravenosen Urographie bei Patienten mit Plasmozytom. Schweiz Med Wschr 1983, 113, 301-304.
58. Lasser EC, Lang JH, Zawadzki ZA. Contrast media. Myeloma protein precipitates in urography. J Am Med Assoc 1966, 198, 273-275.
59. Coleman M, Silver RT. The chemotherapy of plasma cell myeloma and related disorders. Antibiotics and chemotherapy (Karger, Basel) 1974, vol 18, 112-147.
60. Bonnet JD. The management of multiple myeloma and related disorders. In: Carter SK, Goldstein E, Livingston E (eds): Principles of cancer treatment, Mc Graw-Hill, New York 1982, 771-778.
61. Kyle RA, Greipp PR. Smoldering multiple myeloma. N Eng J Med 1981, 302, 1347-1349.
62. Salmon SE, Smith BA. Immunoglobulin synthesis and total body tumor cell number in IgG multiple myeloma. J Clin Invest 1970, 49, 1114-1121.
63. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 1975, 36, 842-854.
64. Durie BGM, Cole PW, Chen HSG, Himmelstein KJ, Salmon SE. Synthesis and metabolism of Bence Jones protein and calculation of tumour burden in patients with Bence Jones myeloma. Brit J Haematol 1981, 47, 7-19.

65. Salmon SE, Wampler SB. Multiple myeloma: Quantative staging and assessment of response with a programmable pocket calculator. Blood 1977, 49, 379-386.
66. Merlini G, Waldenström JG, Jayakar SD. A new improved clinical staging system for multiple myeloma based on the analysis of 123 patients. Blood 1980, 55, 1011-1019.
67. McIntyre W. Case of mollities and fragilitas osseum accompanied with urine strongly charged with animal matter. Med Chir Trans 1850, 33, 211-232.
68. Thomas JJ. A case of myeloma of the spine with compression of the cord. Boston Med Surg 1901, 145, 367-373.
69. Snapper I, Turner LB, Moscovity HL. Multiple myeloma. Grune and Stratton, New York, 1953, pp 124-146.
70. Holland JF, Hosley H, Scharlau, Carbone PP, Frei E, Brindley CO, Hall TC, Schrider BI, Gold GL, Lasagna L, Owens AH, Miller SP. A controlled trial of urethane treatment in multiple myeloma. Blood 1966, 27, 328-342.
71. Feinleib M, MacMahon. Duration of survival in multiple myeloma. J Nat Cancer Inst 1960, 24, 1259-1269.
72. Rivers LR, Whittington RM, Patno ME. Cyclophosphamide treatment in multiple myeloma. Cancer Chemother Rep 1963, 29, 115-119.
73. McArthur JR, Athens JW, Wintrobe MM, Cartwright GE. Melphalan and multiple myeloma. Experience with a low-dose continuous regimen. Ann Intern Med 1970, 665-670.
74. Korst DR, Clifford GO, Fowler MW, Louis J, Will J, Wilson HE. Multiple myeloma. II: Analysis of cyclophosphamide therapy in 165 patients. J Am Med Assoc 1964, 189, 156-160.
75. Alexanian R, Bergsagel DE, Migliore PJ, Vaughn WK, Howe CD. Melphalan therapy for multiple myeloma. Blood 1968, 31, 1-10.
76. Alexanian R, Haut A, Khan AU, Lane M, McKelvey EM, Migliore PJ, Stuckey WJ, Wilson HE. Treatment for multiple myeloma. Combination therapy with different melphalan dose regimens. J Am Med Assoc 1969, 208, 1680-1685.
77. Rivers SL, Patno ME. Cyclophosphamide versus melphalan in treatment of plasma cell myeloma. JAMA 1969, 207, 1328-1334.
78. McArthur JR, Athens JW, Wintrobe MM, Cartwright GE. Melphalan and myeloma. Experience with a low-dose continuous regimen. Ann Intern Med 1970, 665-670.
79. Medical Research Council. Myelomatosis: comparison of melphalan and cyclophosphamide therapy. Brit Med J 1971, I, 640-641.
80. Brook J, Bateman JR, Gocka EF. Long-term low-dose melphalan treatment of multiple myeloma. Arch Int Med 1973, 131, 545-548.
81. Mellstedt H, Björkholm M, Holm G. Intermittent melphalan and prednisone therapy in plasma cell myeloma. Acta Med scand 1977, 202, 5-9.
82. Bergsagel DE, Bailey AJ, Langley GR, McDonald RN, White DF, Miller AB. The chemotherapy of plasma cell myeloma and the incidence of acute leukemia. N Eng J Med 1979, 301, 743-748.
83. Medical Research Council. Report on the second myelomatosis trial after 5 years of follow-up. Br J Cancer 1980, 42, 813-822.
84. Abramson N, Lurie P, Mietlowski WL, Schilling A, Bennet JM, Horton J. Phase III study of intermittent carmustine (BCNU), cyclophosphamide, and prednisone versus intermittent melphalan and prednisone. Cancer Treat Rep 1982, 66, 1273-127
85. McElwain TJ, Powles RL. High dose intravenous melphalan for plasma cell leukemia and myeloma. Lancet 1983, ii, 822-824.

86. Bosanquet AG, Gilby ED. Pharmacokinetics of oral and intravenous melphalan during routine treatment of multiple myeloma. Eur J Cancer Clin Oncol 1982, 18, 355-362.
87. Costa G, Engle RL, Schilling A, Carbone P, Kochwa S, Glidewell. Melphalan and prednisone: an effective combination for the treatment of multiple myeloma. Am J Med 1973, 54, 589-599.
88. Salmon SE, Shadduck RK, Schilling A. Intermittent high-dose prednisone (NSC-10023) therapy for multiple myeloma. Cancer Chem Rep 1967, 51, 179-18
89. Raisz LG, Luben RA, Mundy GR, Dietrich JW, Horton JF, Trummel CL. Effect of osteoclast activating factor from human leucocytes on bone resorption. J Clin Invest 1975, 56, 408-413.
90. George RP, Poth JL, Gordon D, Schrier SL. Multiple myeloma-intermittent combination chemotherapy compared to continuous therapy. Cancer 1972, 29, 1665-1670.
91. Alexanian R, Bonnet J, Gehan E, Haut A, Hewlett J, Lane M, Monto R, Wilson H. Combination chemotherapy for multiple myeloma. Cancer 1972, 30, 382-389.
92. Lee BJ, Sahakian G, Clarkson BD, Krakoff JH. Combination chemotherapy of multiple myeloma with alkeran, cytoxan, vincristine, prednisone and BCNU. Cancer 1974, 40, 533-538.
93. Alexanian R, Salmon SE, Bonnet J, Gehan E, Haut A, Weick J. Combination chemotherapy for multiple myeloma. Cancer 1977, 40, 2765-2771.
94. Case DC, Lee BJ, Clarkson BD. Improved survival times in multiple myeloma treated with melphalan, prednisone, cyclophosphamide, vincristine, BCNU: M-2 protocol. Amer J Med 1977, 63, 897-903.
95. Cohen HJ, Silberman HR, Larsen WE, Johnson L, Bartolucci AA, Durant JR. Combination chemotherapy with intermittent 1-3-bis(2-chloro-ethyl)1-nitrosurea (BCNU), cyclophosphamide and prednisone for multiple myeloma. Blood 1979, 54, 824-836.
96. Harley JB, Pajak TF, McIntyre OR, Kochwa S, Cooper MR, Coleman M, Cuttner J. Improved survival of increased-risk myeloma patients on combined triple alkylating-agent therapy, a study of the CALGB. Blood 1979, 54, 13-22.
97. Cavagnaro F, Lein J M, Pavlovsky S, Becherini JO, Pileggi JE, Micheo EQ, Jait C, Musso A, Suarez A, Pizolatto M. Comparison of two combination chemotherapy regimens for multiple myeloma: methyl-CCNU, cyclophosphamide and prednisone versus melphalan and prednisone. Cancer Treat Rep 1980, 64, 73-79.
98. Alexanian R, Salmon SE, Gutterman J, Dixon D, Bonnet J, Haut A. Chemoimmunotherapy for multiple myeloma. Cancer 1981, 47, 1923-1929.
99. Tirelli U, Crivellari D, Carbone A, Veronesi A, Galligioni E, Trovo MG, Tumolo S, Grigoletto E. Combination chemotherapy for multiple myeloma with melphalan, prednisone, cyclophosphamide, vincristine and BCNU: M-2 protocol. Cancer Treat Rep 1982, 66, 1971-1973.
100. Alexanian R, Dreicer R. Chemotherapy for multiple myeloma. Cancer 1984, 53, 583-588.
101. Buckman R, Cuzick J, Galton DAG. Long term survivors in myelomatosis. Brit J Haematol 1982, 52, 589-599.
102. Gonzalez F, Trujillo JM, Alexanian R. Acute leukemia in multiple myeloma. Ann Intern Med 1977, 86, 440-443.
103. Bergsagel DE. Plasma cell neoplasms and acute leukemia. In: Salmon SE (ed): Clinics in Haematology, WB Saunders Company Ltd, vol 11, no 1, 221-234.
104. Paccagnella A, Cartei G, Fosser V, Salvagno L, Bolzonella S, Sileni VC, Fiorentio MV. Treatment of multiple myeloma with M-2 protocol and without maintenance therapy. Eur J Cancer Clin Oncol 1983, 19, 1345-1351.

105. Bergsagel DE. Phase-II trials of mitomycin-C, AB-100, NSC-1026, L-sarcolysin, and meta-sarcolysin in the treatment of multiple myeloma. Cancer Chemotherapy Rep 1962, 16, 261-266.
106. Costa G, Carbone PP, Gold GL, Owens AH Jr, Miller SP, Krant MJ, Bono VH Jr. Clinical trial of vinblastine in multiple myeloma. Cancer Chemotherapy Rep 1963, 27, 87-89.
107. Carbone PP, Frei E III, Owens AH Jr, Olsen KB, Miller SP. 6-Thioguanine (NSC-725) therapy in patients with multiple myeloma. Cancer Chemotherapy Rep 1964, 36, 59-62.
108. Moon JH, Edmonson JH. Procarbazine (NSC-77213) and multiple myeloma. Cancer Chemotherapy Rep 1970, 54, 245-248.
109. Bergsagel DE, Phil D, Cowan DH, Hasselback R. Plasma cell myeloma: response of melphalan resistant patients to high-dose intermittent cyclophosphamide. Can Med Assoc J 1972, 107, 851-855.
110. Alberts DS, Salmon SE. Adriamycin (NSC-123127) in the treatment of alkylator-resistant multiple myeloma: a pilot study. Cancer Chemotherapy Rep 1975, 59, 345-350.
111. Salmon SE. Nitrosureas in multiple myeloma. Cancer Treat Rep 1976, 60, 789-794.
112. Cohen HJ. Hexamethylmelamine (HMM): a new effective agent in the therapy of refractory multiple myeloma. Haemat Malignancies-Clinical, 1977 (abstract 339), page 18.
113. Bennett JM Silber R, Ezdinli E, Levitt M, Oken M, Bakemeier RF, Bailan JC, Carbone PP. Phase II study of adriamycin and bleomycin in patients with multiple myeloma. Cancer Treat Rep 1978, 9, 1367-1369.
114. Lake-Lewin D, Meyers J, Lee BJ, Young CW. Phase-II trial of pyrazofurin in patients with multiple myeloma refractory to standard cytotoxic therapy. Cancer Treat Rep 1979, 63, 1403-1404.
115. Kyle RA, Gailani S, Seligman BR, Blom J, McIntyre RO, Pajak TF, Holland JF. Multiple myeloma, resistant to melphalan: treatment with cyclophosphamide, prednisone and BCNU. Cancer Treat Rep 1979, 63, 1265-1269.
116. Riccardi A, Merlini G, Montecucco CM. Treatment of multiple myeloma with vincristine. Acta Haematol 1980, 64, 176-178.
117. White D, Bergsagel DE, Rapp EF, Khaliq A, Shelley W, Pater JL. Failure of cyclophosphamide to produce response in melphalan resistant multiple myeloma. Blood 1981, 58, supplement 169a.
118. Brandes LJ, Israels LG. Treatment of advanced plasma cell myeloma with weekly cyclophosphamide and alternate day prednisone. Cancer Treat Rep, 1982, 66, 1413-1415.
119. Bonnet J, Alexanian R, Salmon SE, Bottomley R, Haut A, Amare M, Dixon D. Vincristine, BCNU, doxorubicine and prednisone (VBAP) combination in the treatment of relapsing or resistant myeloma: a Southwest Oncology Group Study. Cancer Treat Rep 1982, 66, 1267-1271.
120. Alberts DS, Durie BGM, Salmon SE. Doxorubicin/BCNU chemotherapy for multiple myeloma. Lancet 1976, i, 926-928.
121. Presant CA, Klahr C. Adriamycin, 1,3Bis(2-chloroethyl)-1-nitrosurea (BCNU, NSC 409962), cyclophosphamide plus prednisone (ABC-P) in melphalan-resistant myeloma. Cancer 1987, 42, 1222-1227.
122. Broun GO jr, Petruska PJ, Hiramoto RN, Cohen HJ. Cisplatin, BCNU, cyclophosphamide and prednisone in multiple myeloma. Cancer Treat Rep 1982, 66, 237-242.
123. Barlogie B, Smith L, Alexanian R. Effective treatment of advanced multiple myeloma refractory to alkylating agents. N Eng J Med, 1984, 310, 1353-1356.

124. Mill WE, Griffith R. The role of radiation therapy in the management of plasma cell tumors. Cancer 1980, 45, 647-652.
125. Durie BGM, Salmon SE. In: Salmon SE (ed): Clinics in Haematology, WB Saunders Company Ltd, London, 1982, vol 11, no 1 ,page 200-201.
126. Jaff JP, Bosch A, Raich PC. Sequential hemibody radiotherapy in advanced multiple myeloma. Cancer 1979, 43, 124-128.
127. Schultz U, Loer F. Die Rolle des Strahlentherapie und der Chirurgie beim ossaren Plasmozytom. Dtsch Med Wschr 1979, 20, 719-721.
128. Kyle RA, Jowsey J. Effects of sodium fluoride, calcium carbonate and vitamin D on the skeleton in multiple myeloma. Cancer 1980, 45, 1669-1674.
129. Cohen HJ, Silberman HR, Tornyos K, Bartolucci AA. Comparison of two long-term chemotherapy regimens, with or without agents to modify skeletal repair, in multiple myeloma. Blood 1984, 63, 639-648.
130. Johnson WJ, Kyle RA, Dahlberg PJ. Dialysis in the treatment of multiple myeloma. Mayo Clin Proc 1980, 55, 65-72.
131. Cosio FG, Pence TV Shapiro FL, Kjellstrand CM. Severe renal failure in multiple myeloma. Clin Nephrol 1981, 15, 206-210.
132. Blumberg A, Burgi W, Marti HR. Plasmapherese Behandlung bei Multiplem Myelom mit Niereninsuffizienz. Schweiz Med Wschr 1983, 113, 398-402.
133. Locatelli F, Dozin C, Pedrini L, Marai P, Di Filippo S, Ponti R, Costanzo R. Steroid pulses and plasmapheresis in the treatment of acute renal failure in multiple myeloma. Proc EDTA 1980, 17, 690-694.
134. Bernstein S, Humes D. Reversible renal insufficiency in multiple myeloma. Arch Intern Med 1982, 142, 2083-2086.
135. Jansen J, Huygens PC, van der Velde EA. The prognosis of multiple myeloma. Neth J Med 1980, 23, 246-251.
136. Matzner Y, Benbassat J, Polliack A. Prognostic factors in multiple myeloma. A retrospective study using conventional statistical methods and a computerprogram. Acta Haematol 1978, 60, 257-268.
137. Medical Research Councils Working Party on leukemia in adults: prognostic features in the third MRC myelomatosis trial. Brit J Cancer 1980, 42, 831-840.
138. Southeastern Cancer Study Group. Treatment of myeloma. Comparison of melphalan, chlorambucil and azathioprine. Arch Intern Med 1975, 135, 157-162.
139. Shustik C, Bergsagel DE, Pruzanski W. Kappa and lambda light chain disease: survival rates and clinical manifestations. Blood 1976, 48, 41-51.
140. Cornell CJ, McIntyre OR, Kochwa S, Weksler DB, Pajak TF. Response to therapy in IgG myeloma patients excreting lambda or kappa light chains. CALGB experience. Blood 1979, 54, 23-29.
141. Norfolk D, Child JA, Cooper EH, Kerruish S, Milford Ward A. Serum β2-microglobulin in myelomatosis: potential value in stratification and monitoring. Br J Cancer 1979, 39, 510-515.
142. Bataille R, Grenier J, Sany J. β2-microglobulin in myeloma: optimal use for staging, prognosis and treatment. A prospective study of 160 patients. Blood 1984, 63, 468-476.
143. Cassuto JP, Krebs BP, Viot G, Dujardin P, Masseyeff R. β2-microglobulin, a tumour marker of lymphoproliferative disorders. Lancet 1978, ii, 108-109.
144. Wutke K, Rüdiger KD, Kelenyi G. Prognose-relevante klinische und morphologische Klassifikation des Multiplem Myeloms. Arch Geschwulstforsch 1979, 49, 671-684.
145. Ludwig H. In: Ludwig H (ed): Multiples Myelom. Springer Verlag Berlin. 1982, 145-146.

146. Mandema E. Over het multipel myeloom, het solitaire plasmocytoom en de macroglobulinaemie. Dissertatie 1956, Groningen, page 149-159.
147. Vercelli D, Di Guglielmo R, Guidi G, Scolari L, Buricchi L, Cozzolino F. Bone marrow percentage of plasma cells in the staging of monoclonal gammopathies. Nouv Fr Hematol 1980, 22, 139-145.
148. Bartl R, Burckhardt R, Fateh-Moghadam A, Gierster P, Bauer-Gell R. The bone marrow histobiopsy as a staging procedure of the routine in monoclonal paraprotein disorders. Hamburg 1979, 5th meeting, Europ and Afric Div Int Soc Haematol.
149. Durie BGM, Salmon SE, Moon TE. Pretreatment tumour mass, cell kinetics and prognosis in multiple myeloma. Blood 1980, 55, 364-372.
150. Woodruff RK, Wadsworth J, Malpas JS, Tobias JS. Clinical staging in multiple myeloma. Brit J Haematol 1979, 42, 199-205.
151. Alexanian R, Balcerzak S, Bonnet JD, Gehan EA, Haut A, Hewlett J, Monto R. Prognostic factors in multiple myeloma. Cancer 1975, 36, 1192-1201.
152. Hobbs JR. Growth rates and responses to treatment in human myeloma. Br J Haematol 1969, 16, 607-617.
153. Durie BGM, Russell DH, Salmon SE. Reappraisal of plateau phase in myeloma. Lancet 1980, ii, 65-68.
154. Durie BGM, Young LA, Salmon SE. Human myeloma in vitro colony growth: Interrelationships between drug sensitivity, cell kinetics and patient survival. Blood 1983, 61, 929-934.
155. Ludwig H. In Ludwig H (ed): Multiples Myelom. Springer Verlag Berlin. 1982, page 172-176.
156. Jänne J, Pösö H, Raina A. Polyamines in rapid growth and cancer. Biochim et Biophys Acta 1978, 473, 241-293.
157. Durie BGM, Salmon SE, Russell DH. Polyamines as markers of response and disease activity in cancer chemotherapy. Cancer Research 1977, 37, 214-221.
158. Drewinko B, Alexanian R. Growth kinetics of plasma cell myeloma. L Natl Cancer Inst 1977, 58, 1247-1253.
159. Bast BJEG, Boom SE, Ballieux RE. Characterization of the colony-forming cell in monoclonal gammopathies. Blood 1982, 60, 608-612.
160. Hamburger A, Salmon SE. Primary bioassay of human myeloma stem cells. J Clin Invest 1977, 60, 846-854.
161. Durie BGM, Salmon SE. Cell kinetic analysis of human tumor stem cells. In: Cloning of human tumor stem cells. Alan R. Liss Inc. New York. 1980, page 153-163.
162. Drewinko B, Brown BW, Humphrey R, Alexanian R. Effect of chemotherapy on the labelling index of myeloma cells. cancer 1974, 34, 526-531.
163. Alberts DS, Durie BGM, Salmon SE. Treatment of multiple myeloma in remission with anticancer drugs having cell cycle specific characteristics. Cancer Treat Rep 1977, 61, 381-388.
164. Drewinko B, Alexanian R, Boyer H, Barlogie B, Rubinow SI. The growth fraction of human myeloma cells. Blood 1981, 57, 333-338.
165. Karp JE, Burk PJ, Humphrey RL. Induction of serum stimulation and plasma cell proliferation during chemotherapy for multiple myeloma. Blood 1977, 49, 925-934.
166. Karp JE, Humphrey RL, Burke PJ. Timed sequential chemotherapy of cytoxan-refractory multiple myeloma with cytoxan and adriamycin based on induced tumor proliferation. Blood 1981, 57, 468-475.

Chapter 2

Vindesine-prednisone treatment of patients with melphalan-resistant multiple myeloma

2.1 Introduction

Multiple myeloma can be treated initially with melphalan and prednisone. This results in objective regression of signs and symptoms and tumour cell mass as well as in a significant increase in survival time, in the majority of patients [1]. In later stages however, the disease frequently becomes resistant against melphalan, though not necessarily to other alkylating agents [2,3]. Cell cycle specific agents such as vincristine have also been effective in multiple myeloma, especially when used in combination with alkylating agents [4,5]. In a small number of patients we observed objective responses with vincristine, in combination with prednisone [unpublished results]. The rapid onset of disabling neurotoxicity appeared to be the major side effect, sometimes even after a single vincristine dose. It was this side effect which was responsible for withdrawal of vincristine in most of our patients, and which led us to consider a new vinca alkaloid: vindesine. Vindesine (desacetyl vinblastine amide sulphate, Eldisine®) is thought to be more or less the intermediate between vincristine and vinblastine as far as toxicity (especially neurotoxicity) is concerned [6]. The clinical value of vindesine has extensively been studied and responses have been reported in non-small cell lung carcinoma [7], testicular cancer [8], chronic myelocytic leukaemia in terminal phase [9] and in breast cancer [10].

In patients with advanced multiple myeloma with disease progression or with symptomatic disease not responding to melphalan-prednisone, a study was done to test the therapeutic and toxic effects of vindesine. Weekly vindesine injections were used on the basis of data obtained in a phase I study [11].

2.2 Patients and methods

Patients

Patients resistant to melphalan-prednisone treatment were consecutively admitted to the study. Resistance to melphalan therapy was defined as 1) progression of symptoms and/or rise of myeloma pro-

tein level, and/or 2) pancytopenia (platelet count below 50×10^9/l, WBC below 1.5×10^9/l) under melphalan-prednisone treatment. Non-written informed consent was obtained from every patient.

Upon entry patients were staged according to the following criteria [12]:

Stage I: haemoglobin > 100 g/l; serum calcium < 3 mmol/l; m-protein level for IgG < 50 g/l, for IgA < 30 g/l; light chain excretion in urine < 4 g/24 h; on skeletal X-ray examination no lesions.

Stage II: fitting neither Stage I nor III.

Stage III: haemoglobin < 85 g/l; serum calcium > 3 mmol/l; m-protein level for IgG > 70 g/l, for IgA > 50 g/l; light chain excretion > 12 g/24 h; multiple skeletal lesions on X-ray examination.

For stage I all criteria had to be met, for stage III one criterion was sufficient. Subclassification A or B depended on normal or impaired renal function (serum creatinine > 180 μmol/l). Laboratory tests were performed upon entry and before every vindesine injection. Skeletal X-ray surveys were performed upon entry and at the time of response evaluation.

Treatment protocol

Vindesine was administered as a bolus injection through a running intravenous infusion. Initially the dose was 3 mg/m^2 body surface, but this caused unacceptable neurotoxicity in the first three patients. The dose was then reduced to 2 mg/m^2. The vindesine was given weekly for three consecutive weeks (one 'course'). These courses were alternated with three-week therapy free intervals. After each vindesine injection prednisone was given orally in a daily dose of 100 mg for 5 days.

Response evaluation

Patients were considered evaluable for response after at least three vindesine injections. Whenever possible response was evaluated after 2 courses of therapy. Response was measured according to the criteria of the Southwest Oncology Group (SWOG, [13]):

'Complete response': disappearance of m-protein and disease activity.

'Partial response': decrease in serum myeloma protein level to less than 25% of the pretreatment value, normalisation of anaemia and hypercalcaemia as well as disappearance of light-chain excretion.

'Improvement': decrease in serum m-protein level to less than 50% of the pretreatment value, with normalisation of anaemia and hypercalcaemia.

'No-response': less than 50% decrease in serum myeloma protein level, without signs of disease progression.
'Progression': increasing serum myeloma protein level and/or progression of skeletal pains, skeletal lesions, hypercalcaemia and anaemia.

2.3 Results

Forty patients entered the study and 34 (twenty males and fourteen females, mean age 61.6 year) were evaluable. Six patients were considered not to be evaluable because they died within three weeks from entering the study (one pneumonia, one myocardial infarction, two unexplained sudden deaths at home, one renal failure due to amyloidosis, one unrelated malignancy). Median duration of initial melphalan-prednisone treatment was 20 months with a range of 2-132 months. In addition to the melphalan-prednisone treatment, 4 patients had been treated with vincristine, 3 with cyclophosphamide, 2 with CCNU and 2 with the M-2 regimen (which consists of vincristine, melphalan, cyclophosphamide, BCNU and prednisone). Data on the clinical classification and on the isotype distribution of the m-proteins are given in Table 1.
Thirty-two patients were having stage III disease corresponding with a high tumour load. Results after two courses of vindesine-prednisone were as follows: a 'partial response' was obtained in five patients (fifteen percent), 'improvement' in four patients (twelve percent) while the majority (eighteen patients 53%) showed 'no-response'. 'Progression' occurred in seven patients (20%). In the patients with a 'partial response' or an 'improvement', treatment with vindesine-prednisone was continued. The duration of response and the final outcome in these patients is shown in Table 2. Four patients died while their myeloma was in a stable condition (3 of bacterial infection and 1 sudden death at home). Four patients (no 1, 2, 6 and 7) showed progressive disease after 30, 18, 19 and 13 weeks, calculated from the

Table 1. M-protein class distribution and disease stage (according to Durie and Salmon) in 34 myeloma patients.

	IgG		IgA		BJ	
	λ	κ	λ	κ	λ	λ
IIA	1		1			
IIIA	5	16	3	4		3
IIIB					1	

Table 2. Duration of response and final outcome in 9 patients.

No.	Duration of response/ improvement (weeks)	End of response indicated by
1	30	disease progression
2	18	disease progression
3	14	died of pneumonia
4	14	died of unknown cause
5	54	still responding
6	19	disease progression
7	13	disease progression
8	12	died of pneumonia
9	42	died of Gram-negative septicaemia.

start of the vindesine-prednisone treatment. One patient (presenting with hypercalcaemia and a serum IgG m-protein level of 96 g/l after melphalan-prednisone treatment) is still being treated with vindesin-prednisone after 2 years.

Toxicity

With a vindesine dose of 3 mg/m^2 severe neurotoxicity was observed and we therefore decreased the dose to 2 mg/m^2. Toxicity consisted of alopecia (fifteen percent of the patients) and fingertip paraesthesias in 50%. Alopecia and paraesthesias were reversible in several patients despite continued treatment. Paralytic ileus or severe constipation were not observed.

Haematological side effects were mild. Leukopenia was infrequently observed with a nadir of 1.1×10^9/l. None of the three responding patients who died from infection was leukopenic at that time.

2.4 Discussion

After treating the first eleven patients the vindesine-prednisone combination obtained a response rate of 55%: two 'partial responses' and four 'improvements' [14]. The next 23 patients thus treated, showed only three 'partial responders', so that the response rate for a total of 34 patients was 26%.

Shortly after the end of this study Alexanian et al. published a paper with the results on vindesine treatment of melphalan-resistant myeloma patients [15]. In this study eleven patients were treated with vindesine alone and no patient showed an objective response. Five of them were relapsing to initial chemotherapy and six were initially unresponsive. All had previously been treated with vincristine. Alex-

anian et al. further treated sixteen patients with the same vindesine-prednisone combination as used in our study, and this resulted in three 'partial responses' plus one 'improvement' (response rate 25%). Suspecting the therapeutic effects of prednisone they then treated sixteen melphalan-resistant patients with prednisone alone. This resulted in a response rate of 31% (three 'partial responses' and one 'improvement'). They then concluded that the benefit of the vincristine-prednisone combination resulted primarily from the frequent courses of large doses of prednisone. However, all of the 'vindesine-only' patients had shown resistance to vincristine, so that a therapeutic effect of vindesine for non-vincristine treated patients is not excluded. In fact, the significant increases of spermidine levels after vindesine administration, in several patients who responded later to the vindesine-prednisone combination (Chapter 6), is an argument for vindesine-induced tumour kill. The response duration in our nine responding patients was short, indicating the rapid development of drug resistance. In the Alexanian study response duration was also short.

In our study a high rate of fatal infections was found: three of the nine responding patients. Alexanian et al. reported a 50% incidence of febrile (39 °C) episodes in his patient population, for which hospitalization was frequently indicated. Two of his patients died of infection, both from bilateral pneumonia. The high infection rate with this vindesine/prednisone combination was probably the result from the high and prolonged use of prednisone, since leukopenia occurs only infrequently.

In conclusion this vindesine-prednisone combination clearly is not a very effective second-line therapy for patients with melphalan-resistant multiple myeloma. The efficacy of vindesine in these patients needs to be further evaluated.

References

1. Durie BGM, Salmon SE. The current status and future prospects of treatment for multiple myeloma. In: Wolvey W editor. Myeloma and related disorders. Eastbourne, WB Saunders Company Ltd. Feb 1982, vol 11, no 1, 181-210.
2. Bergsagel DE, Cowan HE, Hasselback R. Plasma cell myeloma: response of melphalan resistant patients to high-dose cyclophosphamide. Can Med Assoc J, 1972, 107, 851-855.
3. Kyle RA, Gailani S, Seligmann BR et al. Multiple myeloma resistant to melphalan: treatment with cyclophosphamide, prednisone and BCNU. Cancer Treat Rep 1979, 63, 1265-1269.
4. Ricardi A, Merlini G, Montecucco CM. Treatment of multiple myeloma with vincristine. Acta Haemat 1980, 64, 176-178.
5. Alexanian R, Salmon SE, Bonnet J, Gehan E, Haut A, Weich J. Combination chemotherapy for multiple myeloma. Cancer 1977, 40, 2765-2771.

6. Smith IE, Hedley DW, Powles TJ, McElwain TJ. Vindesine: A phase II study in the treatment of breast carcinoma, malignant melanoma and other tumours. Cancer Treat Rep 1978, 62, 1427-1433.
7. Furnas BE, Williams SD, Einhorn LH, Cobleigh MA. Vindesine: an effective agent in the treatment of non-small cell lung cancer. Cancer Treat Rep 1982, 66, 1709-1711.
8. Reynolds TF, Vugrin D, Cvitkovic E, Gralla RJ, Young CW, Galbey RB. Phase II trial of vindesine in patients with germ-cell tumours. Cancer Treat Rep 1979, 63, 1399-1401.
9. Baccarani M, Corbelli G, Zaccaria A, Tura S. Phase II trial of vindesine in leukemia and lymphoma. Periti P, Grassi GG, eds. Proc. 12th Internat Cong Chemotherapy 1981, vol II, 1411-1412.
10. Walker BK, Raich PC, Fontana J, Subramanian VP, Rogers II JS, Knost JA, Denning B. Phase II study of vindesine in patients with advanced breast cancer. Cancer Treat Rep 1982, 66, 1729-1732.
11. Dyke RW, Nelson RL. Phase I anti-cancer agents. Vindesine (desacetyl vinblastine amide sulphate). Cancer Treat Rev 1977, 4, 135-142.
12. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Cancer 1975, 36, 842-854.
13. Bonnet J, Alexanian R, Salmon SE et al. Vincristine, BCNU, doxorubicine, and prednisone (VBAP) combination in the treatment of relapsing multiple myeloma: a Southwest Oncology Group Study. Cancer Treat Rep 1982, 66, 1267-1271.
14. Houwen B, Ockhuizen Th, Marrink J, Nieweg HO. Vindesine therapy in melphalan-resistant multiple myeloma. Eur J Cancer 1981, 17, 227-232.
15. Alexanian R, Yap BS, Bodey GP. Prednisone pulse therapy for refractory myeloma. Blood 1983, 62, 572-577.

Chapter 3

Cyclophosphamide, adriamycin, prednisone and vindesine combination chemotherapy in melphalan-resistant multiple myeloma

3.1 Introduction

Initial treatment of multiple myeloma generally consists of melphalan/prednisone, although other regimes (M-2, BCNU/cyclophosphamide/prednisone [1,2]), are used. These regimes induce a response with relief of symptoms in 50-80% of previously untreated patients. However cure of multiple myeloma is extremely rare and eventually all patients become resistant to treatment, which is heralded by increasing m-protein and/or symptoms (e.g. skeletal pains). In these relapsing patients disease is difficult to control and from a variety of drug regimens a choice has to be made for further treatment.

The aim of such a rescue treatment is to prolong the patients life with as little disease symptoms and drug-induced toxicity as possible. Cyclophosphamide, carmustine, adriamycin and vincristine have therapeutic effects, alone or in combination, and response rates are 0-58% [3-15]. In a trial with vindesine/prednisone we achieved a response rate of 25%, but the duration of the response was short [16,17]. Several patients died from infection.

In non-responding patients or relapsing patients the vindesine/prednisone treatment was extended with cyclophosphamide since cross-resistance with melphalan is not always present[6]. Because this did not result in objective responses, adriamycin was included in the treatment. This induced tumour reduction in several patients, and the four-drug regimen (cyclophosphamide, adriamycin, prednisone and vindesine (Eldisin®: CAPE) was therefore chosen for evaluation in melphalan-resistant myeloma. This paper reports on our experiences with the CAPE regimen in a group of 22 patients.

3.2 Patients and methods

Resistance to melphalan was defined as clinical (skeletal pains/fractures, pancytopenia) and/or biochemical (increasing m-protein, hypercalcaemia) deterioration during melphalan/prednisone treatment. When entering the study all patients were staged according to Durie and Salmon [18]. Stage I (low tumour mass): haemoglobin > 100 g/l,

serum calcium < 3.00 mmol/l. Serum myeloma protein level for IgG < 50 g/l and for IgA < 30 g/l, light chain excretion in urine less than 4 g/24 h. Skeletal X-ray normal or solitary plasmacytoma only. Stage II (intermediate tumour mass): fitting neither stage I nor III. Stage III (high tumour mass): haemoglobin < 85 g/l, serum calcium > 3.00 mmol/l. Serum myeloma protein level for IgG > 70 g/l, for IgA > 50 g/l, light chain excretion in urine > 12 g/24h. Skeletal X-ray: advanced lytic bone lesions. For stage I all criteria had to be met, for stage III one criterion was sufficient. Subclassification A or B depended on normal (A) or impaired (B, serum creatinine > 180 μmol/l) renal function.

Laboratory tests (complete blood count, serum urea/creatinine levels, immunoglobulin levels, Bence Jones protein excretion) were done before entry in the study and before every chemotherapy course. Skeletal X-ray surveys were done upon entry into the study and when clinically indicated.

Therapy protocol

The CAPE regimen consists of cyclophosphamide (500 mg/m^2), adriamycin (25 mg/m^2), prednisone (60 mg/m^2) and vindesine (2 mg/m^2). Cyclophosphamide, adriamycin and vindesine were given intravenously on day I. Prednisone was given orally on day 1-5. In several patients cyclophosphamide was given orally instead of intravenously; in that case the dose (500 mg/m^2) was divided over days 1-5. The drugs were administered every three weeks (day 1 = day 22). In patients with complete or partial response of at least four months duration, the therapy interval was gradually increased (maximum interval six weeks).

Response criteria, survival calculation

Patients were evaluated after three chemotherapy courses. Response to treatment was measured according to the criteria of the Southwest Oncology Group [SWOG, 3]. Complete response was defined as disappearance of the m-protein. Partial response, improvement and no response were defined as a decrease in m-protein by 75-90%, 50-75% and 0-50%, respectively. The decrease in m-protein had to be reproducible on at least two consecutive occasions. Progressive disease was indicated by a significant increase in m-protein and/or symptoms. Median survival was calculated according to Peto et al [19].

Table 1. Individual patient data

Patient	Name	Sex	Age	M-type	Stage	Pretreatment[a] (months)		Relapse indicated by	CAPE[b]	M-protein[c] pre	nadir	Response[d]	Follow-up[e]	Cause of death	Remarks[f]
1	K-S	f	69	BJ-K	IIIA	MP	84	multiple fractures	14	non-exc.		NR	died	sudden death at home	
2	S-Z	f	68	A-λ	IIIA	MP	9	M-protein ↑	3	61-41		NR	died	glioblastoma	
3	F	m	56	BJ-λ	IIIA	MP	15	extensive bone destruction; extra osseous lesions	12	non-exc.		prog.	died	bilat. pneum. aggresive myeloma, dedifferentiated	
4	K-V	f	69	G-K/ BJ-K	IIIA	MP	44	skelet. lesions ↑ BJ-K exc. ↑	18	++++	+++	NR	alive Karn 80		skeletal pain ↓ Hb ↑
5	W-T	f	51	G-λ	IIA	MP	88	M-protein ↑	17	70-40		NR	alive Karn 90		after adriamycin 300 mg/m² no CHF
6	S-H	f	60	BJ-K	IIIA	MP	18	BJ-K ↑ skelet. lesions plasma cells in periph. bl. Hb ↓	11	++++	+++	NR	alive Karn 90		Imp. clin. cond. corr. CBC, disappearance of periph plasma cells
7	K	m	74	A-K	IIIA	MP	12	skelet. lesions ↑ Hb ↓	19	38-11		Imp	alive, resp. after 14 m Karn 60		after adriamycin 500 mg/m² no CHF
8	T	m	73	BJ-K	IIIA	MP	60	skelet. lesions ↑	15	non-exc.		NR	alive Karn 70		
9	M	m	60	G-K	IIIA	MP	9	skelet. fractures, thrombo's ↓	4	36-18		Imp	alive Karn 80		skelet. pain ↓ blood count normal
10	E	m	77	G-K	IIIA	MP	18	pancytopenia with diffuse marrow involv.	7	30-32		prog.	died	septicaemia	
11	t.B	m	47	G-λ	IIIA	MP	47	multiple fractures	5	35-40		prog.	alive Karn 60		stable on methylpredn. + E. in 24 h infusion
12	B-B	f	71	G-K	IIIA	MP EP	21 8	multiple fractures	8	4-4		NR	alive Karn 90		
13	R-M	f	39	G-K	IIIA	MP M2	4 5	skelet. pain ↑	5	24-52		prog.	PR to Vinc/ BCNU/ adr		

14	M	m	63	G-K	IIIA	MP EP EPC	21 3 4	fractures bone marrow 60% plasma c.	7	22-20		NR	pred. alive/well Karn 90		
15	H-O	f	61	G-K	IIIA	MP EP	72 15	M-prot.↑ skelet. le- lesions↑ pancytopenia	25	59-42		NR	alive Karn 70		CHF after 560 mg/m² adriamycin
16	V-B	f	62	G-K	IIIA	MP EP EPC	17 4 7	skelet. pain↑ M-protein↑	20	22-15		NR	alive Karn 80		pain↓
17	W-L	f	66	G-K	IIIA	MP EP EPC	4 2 7	M-protein↑	15	56 8		PR	alive Karn 70		
18	W-L	f	78	A-K	IIIA	MP EP	30 4	M-protein↑	11	38 25		NR	alive Karn 70		CHF after 300 mg/m² adriamycin
19	L-S	f	40	BJ-K	IIIA	MP EP EPC	9 2 10	skelet lesions↑	11	non-exc.		prog.	died	plasmacytoma in every organ	
20	K	m	58	BJ-λ	IIIB	MP EP EPC	3 5 4	hypercalc. B.J.exc.↑ renal func.↓	17	5.9 0.8 (g/24 h)		PR	died	agg. MM + extraosseus lesions	previously CHF + MI; after 345 mg/m² adriamycin no CHF
21	J	m	56	G-λ	IIIA	MP EP	2 12	pancytopenia	1	91 -		n.e.	died	septicaemia	
22	B-K	f	71	G-K+λ	IIIA	MP EP	18 3	skelet. lesions↑	1	41 -		n.e.	died	ventricular fibrillation	

[a] MP = melphalan/prednisone; EP = vindesine/prednisone; EPC = vindesine/prednisone/cyclophosphamide
[b] Number of CAPE courses until evaluation
[c] pre = M-protein level in g/l just prior to CAPE: non-exc. = no M-protein. Myeloma subtype was based on bone marrow immunofluorescence studies, or on electrophoresis data before dedifferentiation; + to ++++ indicates estimation of BJ proteinuria
[d] PR = partial response, Imp. = improvement; NR = no response; prog. = progressive disease; n.e. = not evaluable
[e] Karn = Karnofsky score
[f] CHF = congestive heart failure

3.3 Results

The study population comprised 22 patients (13 females, 9 males) with a median age of 62.5 years (range 39–78 years). Detailed clinical and biochemical data are given in table 1. The duration of initial melphalan/prednisone treatment ranged from 3 to 88 months (median 18 months). The response to initial melphalan/prednisone treatment (SWOG criteria) consisted of five 'improvements' (patients 1, 5, 6, 10 and 14). The remaining patients showed either 'no response' or 'progressive disease'.

Five patients (patients 12, 15, 18, 21 and 22) received vindesine-prednisone and five (patients 14, 16, 17, 19 and 20) received treatment with vindesine-prednisone and vindesine-prednisone-cyclophosphamide before they received the CAPE-regimen. One patients [13] had received the M-2 regimen (which consists of melphalan, prednisone, cyclophosphamide, vincristine and carmustine) between melphalan and CAPE treatment. The remaining 11 patients (1-11) were treated with CAPE from the moment melphalan resistance was observed. Except for patient 5 (having stage IIA disease) and patient 20 (stage IIIB), all patients had stage IIIA disease.

Response

Two patients [21,22] died before they could receive their second CAPE course (cause of death indicated in table 1). They were excluded from response evaluation. Two patients [17,20] showed a 'partial response', two [7,9] and 'improvement', and five (patients 3, 10, 11, 13 and 19) showed 'progressive disease'. The majority showed 'no response' (11 patients). In six of these the serum m-protein decreased with a median of 32.5% (range 10-43%).

Survival

All patients were included in the calculation of the median survival. Survival data on the patients who received vindesine-prednisone and vindesine-prednisone-cyclophosphamide were not different from those on the patients who received CAPE treatment from the moment of melphalan resistance (separate data not shown). The median survival time calculated from the start of CAPE treatment was thirteen months. At the moment of evaluation fourteen of 22 patients were alive.

Gasrointestinal toxicity manifested by transient nausea for 24 hours after chemotherapy occurred in 70 per cent of the patients. Vomiting occurred especially after intravenous administration of cyclophosphamide.

The majority of patients experienced hair loss, ranging from thinning to complete alopecia (six patients). In most patients hair growth returned, but never completely. Despite these effects, none of the patients refused therapy. Neurological toxicity, which could be expected from the use of a vinca alkaloid, was not observed.

Two patients [15,18] developed congestive heart failure after a cumulative adriamycin dose of 560 and 300 mg/m^2, respectively. Adriamycin was discontinued and the heart failure responded to digitalis and diuretics. No indications for amyloidosis were found, but its coexistence cannot be excluded; rectal biopsies were not done. Another patient [20] with mitral insufficiency and a previous period of congestive heart failure showed no cardiac symptoms after 345 mg/m^2 adriamycin (cumulative dose). One patient [22] died of ventricular fibrillation 30 days after the first CAPE course (the second course had been postponed in view of radiotherapy for collapsing vertebrae).

Haematological side effects consisted mainly of leukopenia, which was mild (2.0-2.5 $\times$ 10^9/l) in five patients, moderate (1.0-1.9 $\times$ 10^9/l) in eight patients and severe ($<$ 1.0 $\times$ 10^9/l) in three. Two of the latter category died from septicaemia. Both were pancytopenic due to heavy bone marrow infiltration and previous chemotherapy before CAPE was administered. Trombocytopenia was mild (50-75 $\times$ 10^9/l) in one patient, moderate (25-50 $\times$ 10^9/l) in one and severe ($<$ 25 $\times$ 10^9/l) in one (patient 21).

3.4 Discussion

In contrast to the initial treatment for multiple myeloma, there are at present no universally accepted drug regimens for multiple myeloma resistant to or relapsing after melphalan-prednisone. Over the last 15 years several studies of melphalan-resistant myeloma have been published. Patient numbers in these studies ranged from 9 to 24 and response rates ranged from 0-58 per cent [3-15].

Comparison of response rates is difficult due to differences in response criteria, and several data were not reproducible. For example, Bergsagel et al. [6] reported a 58% response rate in nineteen patients with cyclophosphamide and prednisone, while Mass [7] obtained a

response rate of 22% in nine patients with cyclophosphamide. To our knowledge only one preliminary report (42 patients) has been followed by publication of the results obtained in a large patient population [3,13]. These reports concern treatment of melphalan-resistant patients with a combination of vincristine, BCNU, adriamycin and prednisone (VBAP).

We report on our experience with CAPE. This CAPE regimen resembles the cyclophosphamide, adriamycin, prednisone and vincristine regimen advocated by Alexanian [20] for treatment of newly diagnosed multiple myeloma. Although our first patients were treated in a stepped-up fashion (see Results) we discuss the results for all patients as one group, because there was no difference in response rates and survival times between the patients on an escalating regimen and those who received CAPE from the moment of relapse. The CAPE effects are compared with the VBAP study.

The median survival for VBAP was 7.6 months, versus 13 months for CAPE. CAPE induced responses in 4/20 evaluable patients (2 'partial responses' and two 'improvements') and VBAP in 29/122 patients (5 'complete responses', 20 'partial responses' and 14 'improvements'). Progressive disease was observed in 63/122 patients on VBAP and 5/20 patients on CAPE. A difference was also observed in the 'no response' patients: 20/122 on VBAP and 11/20 on CAPE. Since all patients had disease progression before CAPE, these data indicate that CAPE stabilized the disease in some 50% of our patients, and many experienced relief of symptoms (indicated in Table 1).

In the VBAP study nearly all responses were seen in patients who had shown a response (more than 75% m-protein level reduction) to melphalan-prednisone treatment. Of the five patients in our study who had shown a response to melphalan-prednisone, four showed 'no response' and one 'progressive disease' after CAPE.

Cyclophosphamide did not result in significant tumour reduction in the patients who received EPC before CAPE. In two of these patients adriamycin induced a partial response. The role of cyclophosphamide in the treatment of melphalan-resistant myeloma remains unclear.

Toxic effects of CAPE were mainly alopecia and nausea. Two patients died from septicaemia, after one and seven courses, respectively. Patients with a diffusely and heavily infiltrated bone marrow should be closely watched for bone marrow depression, especially after the first course. Although CAPE can easily be administered on an out-patient basis, it is safer to hospitalize the patients for their first course.

A special problem is the adriamycin related cardiotoxicity. Congestive heart failure can generally be expected after a cumulative adria-

mycin dose of 550 mg/m^2 [2,21]. In the VBAP study (employing the same adriamycin dose as CAPE) no congestive heart failure was observed in 122 patients. We observed congestive heart failure in two patients. Careful screening for cardiac problems seems advisable when the cumulative adriamycin dose approaches the 400 mg/m^2 range. The death caused by ventricular fibrillation in one patient is not necessarily adriamycin-related, since arrhythmias are most frequently seen during or shortly after adriamycin infusion.

The aim of treatment in these relatively old patients is prolongation of life in a reasonable condition at the cost of acceptable drug-induced toxicity. In view of the median survival of 13 months we suggest that CAPE could be a suitable therapy for these patients. A trial in a larger number of patients seems justified.

References

1. Tirelli U, Crivellari D, Carbone A, Veronesi A, Galligioni E, Trovo MG, Tumolo S, Grigoletto E. Combination chemotherapy for multiple myeloma with melphalan, prednisone, cyclophosphamide, vincristine and carmustine (BCNU): M-2 protocol. Cancer Treat Rep 1982, 66, 1971-1973.
2. Abramson M, Lurie P, Mietlowski WL, Schilling A, Bennet JM, Horton J. Phase III study of intermittent carmustine (BCNU), cyclophosphamide and prednisone versus intermittent melphalan and prednisone in myeloma. Cancer Treat Rep 1982, 66, 1273-1277.
3. Bonnet J, Alexanian R, Salmon SE, Bottomley R, Haut A, Amare M, Dixon D. Vincristine, BCNU, doxorubicin and prednisone (VBAP) combination in the treatment of relapsing multiple myeloma: A Southwest Oncology Group study. Cancer Treat Rep 1982, 66, 1267-1271.
4. Alberts DS, Durie BGM, Salmon SE. Doxorubicin/BCNU chemotherapy for multiple myeloma in relapse. Lancet 1976, i, 926-928.
5. Brandes LJ, Israels LG. Treatment of advanced plasma cell myeloma with weekly cyclophosphamide and alternate-day prednisone. Cancer Treat Rep 1982, 66, 1413-1415.
6. Bergsagel DE, Cowan DH, Hasselback R. Plasma cell myeloma: response of melphalan-resistant patients to high-dose intermittent cyclophosphamide. Can Med Assoc J 1972, 107, 851-855.
7. Mass RE. High dose cyclophosphamide vs CCNU treatment of melphalan resistant myeloma. Proc Am Assoc Cancer Res 1976, 17, 250.
8. Western Cancer Study Group. Sequential therapy compared with combination chemotherapy in multiple myeloma. Arch Intern Med 1975, 135, 163-171.
9. Tornyos K, Silverman H, Solomon A. Phase II study with methyl CCNU plus prednisone in previously treated alkylating resistant myeloma. Cancer Treat Rep 1977, 61, 785-787.
10. Salmon SE. Nitrosureas in multiple myeloma. Cancer Treat Rep 1976, 60, 789-794.
11. Cohen HJ, Abramson N, Bartolucci A, Bailar J. BCNU, cyclophosphamide and prednisone versus melphalan and prednisone in myeloma. Proc Am Assoc Cancer Res 1976, 17, 280.

12. Alberts DS, Salmon SE. Adriamycin in the treatment of alkylator-resistant multiple myeloma: a pilot study. Cancer Chemother Rep 1975, 59, 345-350.
13. Bonnet SD, Alexanian R, Salmon SE. Vincristine, BCNU, adriamycin, prednisone combination for treatment of melphalan and cytoxan resistant multiple myeloma. Proc Am Assoc Cancer Res 1977, 18, 343.
14. Bennett JM, Silver R, Erdinli E, et al. Phase II study of adriamycin and bleomycin in patients with multiple myeloma. Cancer Treat Rep 1978, 62, 1367-1369.
15. Lake-Lewin D, Meyers J, Lee BJ, Young CW. Phase II-trial of pyrazofurin in patients with multiple myeloma refractory to standard cytotoxic therapy. Cancer Treat Rep 1979, 63, 1403-1404.
16. Houwen B, Ockhuizen Th, Marrink J, Nieweg HO. Vindesine therapy in melphalan-resistant multiple myeloma. Eur J Cancer 1981, 17, 227-232.
17. Van Dobbenburgh OA, Houwen B, Marrink J, Ockhuizen Th, Nieweg HO. Progress report on vindesine/prednisone treatment of melphalan resistant multiple myeloma. Eur J Cancer Clin Oncol 1983, 19, 861-862.
18. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting features, response to treatment. Cancer 1975, 36, 842-854.
19. Peto R, Pike ML, Armitage P, Breslow ME, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 1977, 35, 1-39.
20. Alexanian R. Treatment of multiple myeloma. Acta Haematol (Basel) 1980, 63, 237-240.
21. Von Hoff DD, Rozencweig M, Piccart M. The cardiotoxicity of anticancer agents. Semin Oncol 1982, 9, 23-33.

Chapter 4

β2-microglobuline in multiple myeloma: limited prognostic significance and minimal correlation with tumour mass

4.1 Introduction

β2-microglobulin (β2-m) is a small polypeptide (molecular weight 11,800), which is part of the Class I HLA molecule and is found on the cell membrane of all nucleated cells. Its amino acid sequence shows a 30% homology with the third constant domain (C_H3) of the IgG heavy chain [1]. Some β2-m is shed into the circulation, possibly as a result of membrane turnover or cell death, and can readily be quantified in biological fluids by a radioimmunoassay [2].

β2-m is excreted by glomerular filtration and subsequently almost completely reabsorbed by the proximal tubule where degradation takes place. Hence, serum β2-m levels are strongly dependent on renal function, while urinary levels are predominantly influenced by tubular function [2]. Apart from decreased glomerular filtration increased β2-m serum levels have been reported in a number of non-malignant conditions, including rheumatoid arthritis and systemic lupus erythematodes [3].

Diseases with increased cell turnover, i.e. malignancies, may be expected to cause high serum levels, which appears to be true for Hodgkin's disease, non-Hodgkin lymphomas and chronic lymphocytic leukaemia [4,5,6]. In contrast to this, in both gastric carcinoma and bronchial carcinoma correlation between tumour mass and β2-m level was absent [7,8].

Some recent studies suggest an important role for β2-m serum levels in the staging and monitoring of patients with multiple myeloma (MM) [9-14]. Serum β2-m levels at the time of diagnosis were reported to correlate with Total Body Myeloma Cell mass (TBMC), haemoglobin concentration, extent of skeletal lesions and, most importantly, with survival. Two reports even suggested that determination of β2-m alone may be a reasonable and convenient alternative for conventional staging [11,14]. Theoretically, serial β2-m determinations are expected to be informative in patients in whom the myeloma cell mass cannot be readily assessed (e.g. Bence Jones MM or non-secreting MM).

To evaluate the clinical significance of serum β2-m determinations in MM, we reviewed the data of 108 consecutive patients, treated at

our hospital between 1961 and 1983. We investigated the correlation of serum β2-m levels with presenting features and with disease stage, as well as the prognostic value of serum β2-m in terms of patient survival. Furthermore, changes in β2-m levels were compared with changes in TBMC, in order to assess the value of β2-m for monitoring disease.

4.2 Patients and methods

Patients

The clinical records of all patients with a monoclonal protein referred to our hospital since 1961, were reviewed. To allow for a clinically meaningful observation period and to improve the accuracy of discriminating MM from Monoclonal Gammopathies of Undetermined Significance (MGUS), patients diagnosed after 1979 were not included in the analysis, yielding a potential follow-up period of at least four years for each patient.

Diagnoses of MM, MGUS and solitary plasmacytoma were made according to established criteria [15]. Of a total of 232 patients with a monoclonal immunoglobulin (IgM excluded) eleven could not be studied because of insufficient data. Ten patients with MGUS and seven patients with solitary plasmocytoma progressed to MM during the study period and they were not included in the analysis. From 108 patients with MM, 87 stored sera samples from the time of diagnosis were available, and from 96 MGUS patients 85 sera were present. The actual study population thus consisted of 87 MM and 85 MGUS patients.

The following data were analyzed: age, body weight, body surface area and Karnofsky Performance Score (KPS); serum levels of creatinine, calcium and albumen; complete blood cell count; qualitative and quantitative analysis of serum and urine monoclonal proteins; extent of skeletal lesions.

Staging at the time of diagnosis was done using the staging system of Durie and Salmon [16]. Stage and classification of the monoclonal protein of the MM patients is shown in Table 1. Calculation of initial and sequential TBMC was done with the formulas of Salmon and Wampler [17].

Treatment consisted of intermittent melphalan/prednisone courses in 75 patients. Twelve patients had been treated with continuous low-dose melphalan.

Table 1. Myeloma type and disease stage (Durie/Salmon staging system) for 87 patients.

		I		II		III	
		A	B	A	B	A	B
IgG	K	11	1	10	3	17	0
	L	4	0	5	1	5	1
IgA	K	3	0	3	0	2	0
	L	3	0	1	0	7	0
BJ*	K	0	0	2	0	4	3
	L	0	1	0	0	0	0
Total		21	2	21	4	35	4

* BJ = Bence Jones ('light chain') myeloma.

Methods

Initial and serial β2-m determinations were retrospectively performed in stored sera (–20 °C). Levels have been shown to be stable under this condition, even after long periods [2]. β2-m levels were determined by radioimmunoassay (Phadebas β2-microtest®, Pharmacia, Uppsala, Sweden). To eliminate the effect of renal function impairment on serum β2-m, the measured β2-m was divided by a predicted value calculated (β2-m CTD) from the serum creatinine level, resulting in a measured/calculated ratio: β2-m M/CTD.

The predicted value can be obtained by employing an empiric formula derived by Cassuto et al. [18]:

$$\log_e \beta2\text{-m} = 3.834 - 5.96Y + 2.94Y^2 - 0.476Y^3 + 0.0252Y^4$$

where $Y = \log_e$ serum creatinine (mg/ml)

This formula is based on analysis of β2-m levels in 795 patients with varying loss of renal function in the absence of malignancy.

Statistics

For correlation of presenting features with β2-m levels linear regression analysis was used. For differences between groups the Wilcoxon ranksum test was employed. For the analysis of two by two contingency tables the chi-square was used. For comparison of survival patterns in different groups the logrank test was used [19]. To examine influences of several presenting features on patient survival, re-

gression analysis based on the model of proportional hazard function was used [20].

4.3 Results

Relationship of β2-m and β2-m M/CTD with disease stage, presenting features and TBMC

β2-m values tended to be somewhat higher in patients with stage IIA or IIIA myeloma then in stage IA (Fig. 1). There was however a considerable overlap. Correction of β2-m for renal function (β2-m M/CTD) yielded similar results (Fig. 2). The overlap precluded accu-

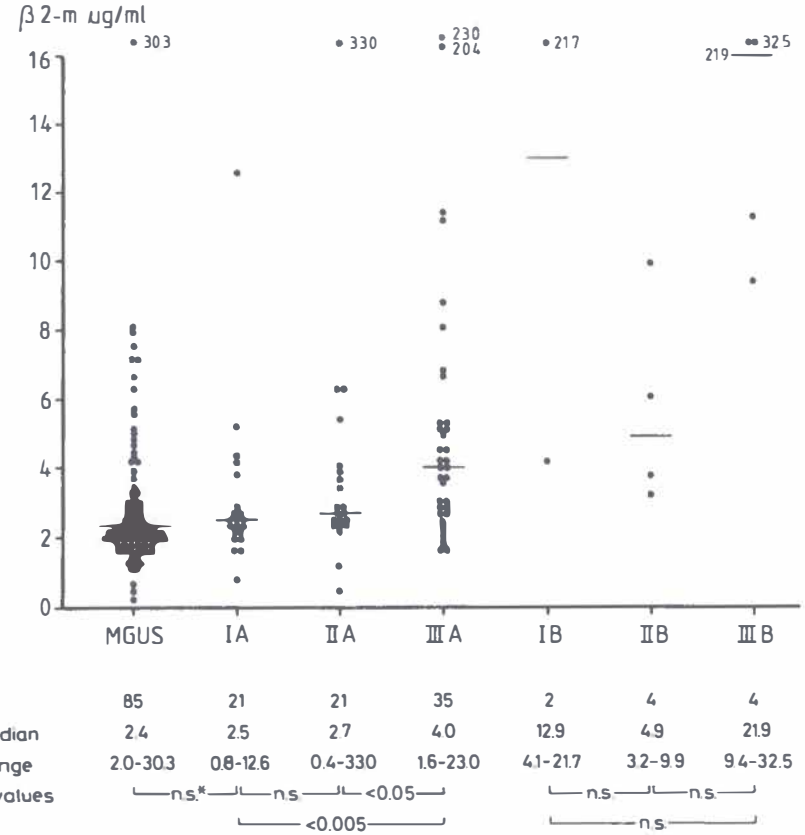

Fig. 1. β2-m levels for 85 MGUS patients and 87 MM patients. The MM patients are subdivided according to their disease stage (Durie/Salmon). Horizontal bars indicate median values.
* = not significant.

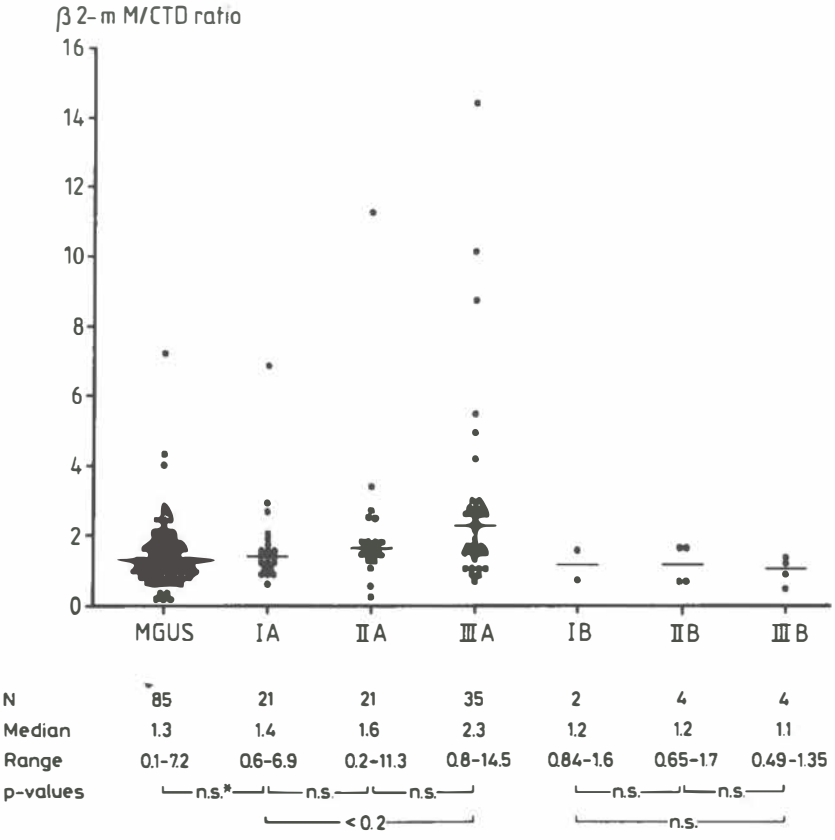

Fig. 2. β2m M/CTD ratios for 85 MGUS patients and 87 MM patients. The MM patients are subdivided according to their disease stage (Durie/Salmon). Horizontal bars indicate median values.
* = not significant

rate prediction of disease stage on the basis of β2-m or β2-m M/CTD in individual patients, as confirmed by decision matrices. Only a β2-m M/CTD > 4.0 indicated patients with stage IIIA (specificity 96%). The sensitivity of this cut-off point, however, was only 17%. Determination of β2-m and β2m M/CTD was of no value in discriminating MGUS from MM stage IA since there was no significant difference between these two groups (Figs. 1 and 2).

Correlations between several presenting features and β2-m are summarized in Table 2. As expected, the strongest correlation was with serum creatinine, but even after correction for renal function, correlations with IgG, TBMC and haemoglobin level remained in the significant range.

Table 2. Correlation between β2-m and β2-m M/CTD and several features at the time of diagnosis.

	β2-m		β2-m M/CTD	
	r''	p	r	p
Haemoglobin	-0.39	<0.001	-0.29	<0.002
Serum calcium	0.36	<0.001	0.04	n.s.*
Albumen level	-0.055	n.s.	-	-
IgG (in IgG MM)	0.33	<0.01	0.27	<0.05
IgA (in IgA MM)	0.41	n.s.	-	-
TBMC	0.28	<0.02	0.26	0.02
KPS	-0.29	<0.01	-0.07	n.s.
Serum creatinine	0.68	≪0.001	-	-

'' = Pearson correlation coefficient.
* = not significant.

Survival analysis

β2-m levels at the time of diagnosis were of evident prognostic significance. The retrospectively determined optimal 'cut-off' value was 2.9 μg/ml. Patients with a β2-m < 2.9 μg/ml had a median survival of 65 months, whereas patients with higher values had a median survival of 25 months ($p < 0.005$, Fig. 3). The group with a β2-m < 2.9 μg/ml consisted primarily of stage IA and stage IIA patients, in contrast to the predominance of IIIA and 'B' patients in the second group (Table 3). This difference was statistically significant, $p < 0.01$. At a level of 6.0 μg/ml we did not find significant survival difference, in contrast to Bataille et al. [11,14].
Similar analysis after correction for renal function (β2-m M/CTD) yielded an optimal 'cut-off' point of 2.0 ($p < 0.05$), which discriminated groups with median survivals of 17.5 and 43 months (data not shown). When these optimal cut-off points (2.9 μg/ml for β2-m and 2.0 for β2m M/CTD) were used within the different disease stages,

Table 3. Distribution of 87 MM patients for disease stage, after stratification for a β2-m level of 2.9 μg/ml (see Fig. 3).

	β2-m < 2.9 μg/ml	β2-m > 2.9 μg/ml
IA	16 (42%)	5 (10%)
IIA	12 (32%)	9 (18%)
IIIA	10 (26%)	25 (51%)
I/II/IIIB	0	10 (21%)

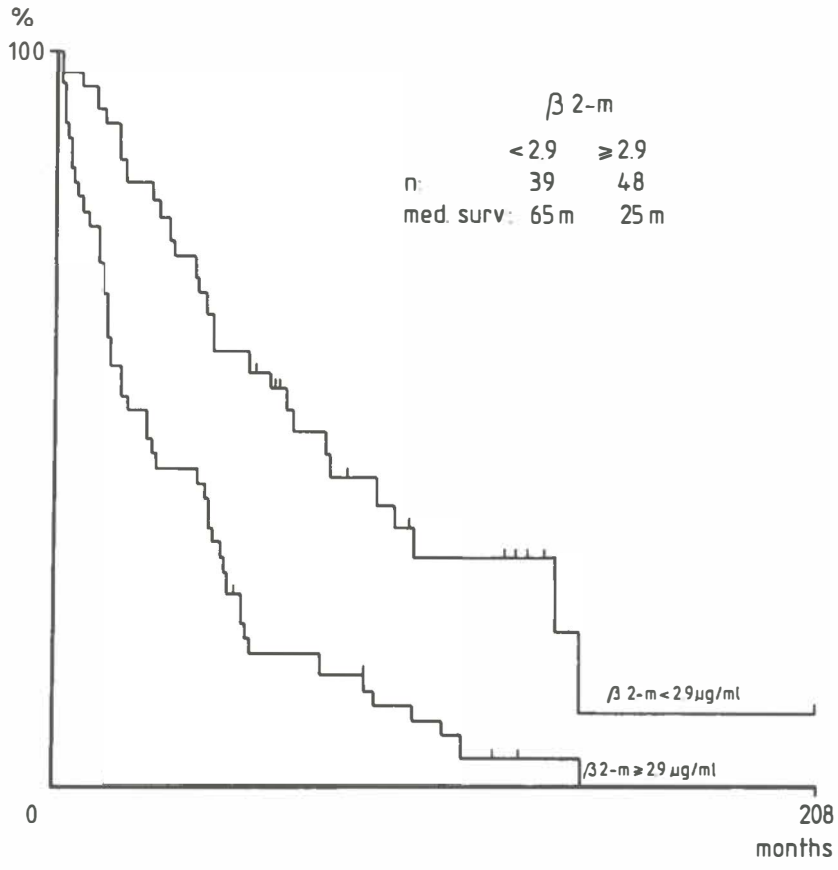

Fig. 3. Survival curves of 87 MM patients after stratification for a β2-m level below or above 2.9 μg/ml.

only stage IIIA could be subdivided by a β2-m M/CTD ratio of 2.0. The resulting subgroups had median survival times of 14 months ($n = 19$) versus 41 months ($n = 19$), ($p < 0.05$, data not shown).

If the serum creatinine was used as a prognostic indicator, the optimal 'cut-off' point was 1.9 mg/100 ml ($p \ll 0.0005$), which is in agreement with the 2.0 mg/100 ml used in the Durie/Salmon staging system.

Multivariate analysis with six presenting features (age, haemoglobin level, TBMC, serum calcium level, β2-m and serum creatinine level) showed that β2-m, haemoglobin level and serum calcium level were without additional influence on survival duration. The serum creatinine level was the most powerful prognostic denominator.

Correlation between changes in β2-m and β2-m M/CTD and changes in TBMC

TBMC reevaluations were performed at 6 and 12 months after the start of therapy. Stored serum samples were available from the 6-month reevaluation of 24 patients and from the 12-month reevaluation of 13 patients. With these data we tested the hypothesis that changes in TBMC are reflected in changes in β2-m.

No correlation could be demonstrated between change in β2-m (expressed as the ratio second β2-m/initial β2-m) and change in TBMC (expressed as the ratio second TBMC/initial TBMC), $p < 0.1$, (Fig. 4). Correction for renal function (β2-m M/CTD) gave similar results, $p < 0.2$, (data not shown). The direction of change of the β2-m ratio did not even tend to correspond to the direction of the TBMC ratio ($p < 0.60$ by chi-square test).

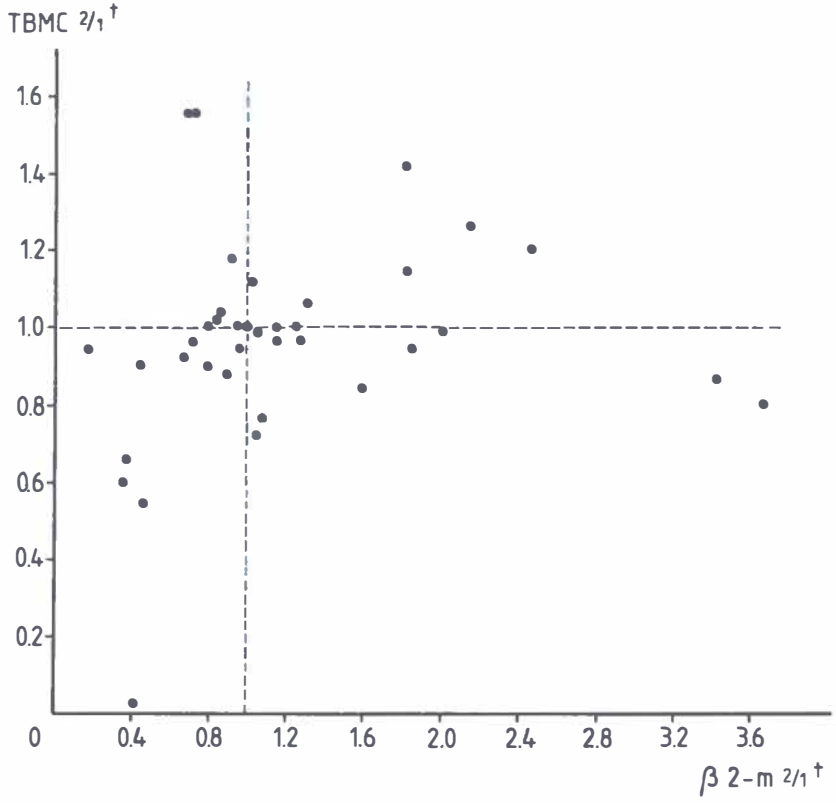

Fig. 4. Correlation of changes in β2-m level with changes in TBMC.
† indicates ratio of second TBMC (or β2-m) and first TBMC (β2-m).

4.4 Discussion

In MM much effort has been dedicated to the search for prognostic parameters. Impaired renal function and anaemia independently influence survival [21]. The staging system of Durie and Salmon includes renal function and haemoglobin level and its stratifying power has been well documented in two large series of patients [22,23]. It has also been recognized however, that staging is of limited value in predicting survival in the individual patient, due to the predominant significance of tumour-drug-sensitivity. Staging systems would improve considerably when a drug sensitivity assay could be included. Unfortunately, as yet no such assay is available for routine practice.

Recently, β2-m levels were reported to be of strong prognostic value. The physiologic basis for this prognostic value has not been clarified and therefore, probably did not receive very much attention. In any case there are no data indicating that β2-m levels have a predictive value for tumour-drug-sensitivity. β2-m levels were also reported to correlate strongly with initial TBMC[13,14] as well as with subsequent changes in TBMC. In our opinion it is not conceivable that β2-m levels would be more useful for TBMC follow-up then the excellent parameter, the monoclonal immunoglobulin, already available in the majority of myeloma patients.

In the present study we found a strong correlation between serum creatinine levels and β2-m levels. Weaker, but still significant correlations were observed between β2-m levels and haemoglobin levels, serum calcium levels, IgG levels in IgG-myeloma and TBMC. In contrast with this, Bataille et al. found the strongest correlation between β2-m and TBMC [11]. In another study Bataille et al. [13] found the correlation between β2-m and TBMC as strong as the correlation between β2-m and haemoglobin level. In our study, the p-values for these correlations were less impressive. The reason for these conflicting results remains obscure.

After correction for renal function (β2-m M/CTD ratio) we found no change in the correlation with TBMC. This was not unexpected since a correlation between TBMC and serum creatinine never has been shown (in the present study the correlation between TBMC and serum creatinine was not significant, $r + 0.17$, $p > 0.1$). For the Durie/Salmon stages we obtained overlapping β2-m values, excluding the possibility of using β2-m values instead of the D/S classification, for instance in non-secreting myelomas. β2-m M/CTD ratios > 4.0 belonged predominantly to stage IIIA, but below this value the overlap precluded any prediction as to the disease stage. These data point to a rather weak correlation between β2-m and tumour load.

Four studies are available with data on survival analysis. Bataille et al. [11,14] used only uncorrected β2-m levels. At a level of 6.0 μg/ml two groups with clearly different survival were separated (maximum follow-up 36 months). Norfolk et al. [10] noticed a different survival at an arbitrarly chosen level of 4 μg/ml in 37 patients. Child et al. [12] found that patients with an uncorrected β2-m > 12 μg/l survived shorter than patients with a β2-m > 2.5 μg/l. In the present study a β2-m level of 2.9 μg/ml had the strongest stratifying potential. In all studies the same Phadebas radioimmunoassay for β2-m was used, so that these different levels are not easily explained, and in fact cause some concern for the design and execution of future prospective studies.

Our multivariate analysis disclosed that the prognostic potential of β2-m disappeared completely after correction for TBMC, serum creatinine level and age. This agrees with the strong correlation between serum creatinine level and β2-m and with the moderate correlation between TBMC and β2-m. The strong influence of renal function on β2-m levels was not only shown in the present study, but had already been reported by other investigators. Indeed, the β2-m level was found to be a more sensitive instrument for detecting moderate changes of glomerular filtration rate than serum creatinine [24]. It is therefore possible that, after correction for serum creatinine (according to Cassuto), some correlation with glomerular filtration rate remains, explaining the moderate prognostic significance of β2-m M/CTD ratios.

We were able to compare changes in TBMC with changes in β2-m levels in 37 patients. If β2-m were indeed a reliable tumour marker in MM, changes in β2-m should at least parallel changes in TBMC. This however was not the case since correlation between these two parameters could not be demonstrated.

In conclusion, we did not find additional stratifying potential of β2-m levels in MM. However, one could argue to use serum β2-m levels instead of serum creatinine levels, TBMC and Durie/Salmon classification. But since these data are already obtained and informative in routine practice and since we did not observe value for β2-m in assessing changes in tumour load, we do not believe there is reason to use β2-m levels in the management of multiple myeloma.

References

1. Berggärd B, Björck L, Cigen R, Lögdberg L. β2-microglobulin. Scand J Clin Lab Invest 1980, 40, suppl 154, 13-24.
2. Karlsson FA, Wibell L, Evrin PE. β2-microglobulin in clinical medicine. Scand

J Clin Lab Invest 1980, 40 suppl 154, 27-37.
3, Shuster J, Gold P, Poulik MD. β2-microglobulin levels in cancerous and other disease states. Clin Chim Acta 1978, 67, 307-313.
4. Child JA, Späti B, Illingworth S, Barnard D, Corbett S, Simmons AV, Stone J, Worthy TS, Cooper EH. Serum beta2-microglobulin and C-reactive protein in the monitoring of lymphomas. Cancer 1980, 45, 318-326.
5. Anderson H, Scarffe JH, Swindell R, Crowther D. Serum β2-microglobulin in patients with non-Hodgkin's lymphoma. Eur J Cancer Clin Oncol 1983, 19, 327-331.
6. Späti B, Child JA, Kerruish SM, Cooper EH. Behaviour of serum β2-microglobulin and acute phase reactant proteins in chronic lymphocytic leucemia. Acta Haemat 1980, 64, 79-86.
7. Staab HJ, Anderer FA, Hiesche K, Wehrle E, Rodatz W. Is serum β2-microglobulin a tumour marker in gastrointestinal cancer? Clin Chim Acta 1980, 106, 309-17.
8. Hällgren R, Nou E, Lundqvist G. Serum β2-microglobulin in patients with bronchial carcinoma and controls. Cancer 1980, 45, 780-5.
9. Scarffe JH, Anderson H, Palmen MK, Crowther D. Prognostic significance of pretreatment serum β2-microglobulin levels in multiple myeloma. Eur J Cancer Clin Oncol 1983, 19, 1361-1364.
10. Norfolk D, Child JA, Cooper EH, Kerruish S, Milford Ward A. Serum β2-microglobulin in myelomatosis,potential value in stratification and monitoring. Br J Cancer 1979, 39, 510-515.
11. Bataille R, Durie BGM, Grenier J. Serum β2-microglobulin and survival in multiple myeloma, a simple reliable marker for staging. Br J Haemat 1983, 55, 439-447.
12. Child JA, Crawford SM, Norfolk DR, O'Quigley J, Scarffe JH, Struthers LPL. Evaluation of serum β2-microglobulin as a prognostic indicator in myelomatosis. Br J Cancer 1983, 47, 111-114.
13. Bataille R, Magub M, Grenier J, Donnadios D, Sany J. Serum β2-microglobulin in multiple myeloma, relation to presenting features and clinical status. Eur J Cancer Clin Oncol 1983, 19, 1075-1078.
14. Bataille R, Grenier J, Sany J. β2-microglobulin in myeloma: optimal use for steging, prognosis and treatment. A prospective study of 160 patients. Blood 1984, 63, 468-476.
15. Committee of the chronic leucemia/myeloma task force National Cancer Institute. Proposed guidelines for protocol studies.II.Plasma cell myeloma. Cancer Chemother Rep 1973, 4, 145-158.
16. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Cancer 1975, 36, 842-854.
17. Salmon SE, Wampler SB. Multiple myeloma, quantative staging and assessment of response with a programmable pocket calculator. Blood 1977, 49, 379-389.
18. Cassuto JP, Krebs BP, Viot G, Dujardin P, Masseyeff R. β2-microglobulin, a tumour marker of lymphoproliferative disorders. Lancet 1978, ii, 108-109.
19. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG: Design and analysis of randomised clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 1977, 35, 1-39.
20. Cox DR. Regression models and life tables. J Royal Stat Soc 1972, 34, 187-220.
21. Medical Research Council. Prognostic factors in the third MRC myelomatosis trial. Br J Cancer 1980, 42, 831-840.

22. Woodruff RK, Wadsworth J, Malpas JS, Tobias JS. Clinical staging in multiple myeloma. Br J Haematol 1979, 42, 199-205.
23. Alexanian R, Balcerzak S, Bonnet JD, Gehan EA, Haut A, Hewlett JS, Monto RW. Prognostic factors in multiple myeloma. Cancer 1975, 36, 1192-1201.
24. Kult J, Lämlein Ch, Röckel A, Heidland A. β2-microglobulin im Serum – ein Parameter des Glomerulus Filtrates. Dtsch Med Wschr 1974, 99, 1636-1641.

Chapter 5

Multiple myeloma with high tumour mass: Treatment with a regimen designed on the basis of cytokinetic data

5.1 Introduction

Treatment of multiple myeloma generally consists of melphalan/prednisone. This therapy results in a response in 40-72% of the patients [1]. In order to obtain greater response rates and longer median survival times, other drug regimens have been tested [1,2]. At the cost of greater drug-induced toxicity this has resulted in moderate increases in response rate and survival times [1,2].

Since these regimens consisted of randomly combined chemotherapeutic agents, we were interested in evaluating the effect of a regimen which was designed on the basis of recent information on cytokinetic behaviour of multiple myeloma. From cytokinetic studies it appears that the tritiated thymidine labelling index (LI) of newly diagnosed, untreated multiple myeloma is low, usually $< 3\%$ [3,4]. Treatment with alkylating agents results in a decrease of Total Body Myeloma Cell number (TBMC) with – in a proportion of patients – an increase in LI, especially within the first months of treatment [4,5]. After initial TBMC reduction many patients appear to enter a stable plateau phase characterized by a low TBMC and a low LI.

However, a substantial proportion does not enter a plateau phase, but shows an unstable disease with a considerable elevated LI [6]. It is possible that these patients were earlier described as having a rapid disease relapse after a rapid initial tumour reduction [7,8]. The increase in LI is thought to reflect recruitment of resting viable myeloma (stem) cells. Karp et al. reported that treatment with cyclophosphamide resulted in a humoral stimulatory activity (HSA) which could be an important cause for the increase in LI [9]. The peak of this HSA occurred nine days after cyclophosphamide administration [10]. In addition to inducing HSA, cyclophosphamide has been shown to be as effective in myeloma as melphalan [11,12].

Vinca alkaloids are especially effective in proliferating cells, and already showed usefulness for treatment of myeloma [2,13,14,15]. Glucocorticoids are employed in almost every regimen for treatment of multiple myeloma. Whether the frequently used dose of 60 mg/m^2 body surface area is really tumouricidal is not known. A dose of 200

mg every other day (as single agent therapy) resulted in significant m-protein level reduction in 8 of 10 patients [16]. High dose methylprednisolone resulted in tumour lysis in high-grade non-Hodgkin lymphomas, and in tumour reduction in Waldenströms disease (unpublished results).

On the basis of the foregoing data we decided to combine cyclophosphamide (C), vindesine (Eldisine®, E) and high dose methylprednisolone (Solumedrol®, S) into a chemotherapeutic regimen (CES, see treatment regimen). The vindesine was given 7 days after the cyclophosphamide/solumedrol in order to make use of the cyclophosphamide induced HSA and other possible mechanisms of cell recruitment. The aim was twofold: to induce profound tumour reduction and to prevent early relapse.

Multiple myeloma patients with a high tumour mass (stage III according to Durie and Salmon, [17]) have a worse prognosis than patients with a low tumour mass [18]. We therefore treated six consecutive patients with stage III disease with CES.

5.2 Patients and methods

Laboratory data

Isotype determination of the myeloma protein was done by immunoelectrophoresis. Subclass analysis of the IgG m-proteins was performed with the Ouchterlony immunodiffusion technique [19], using antisera specific for subclasses from the Red Cross Blood Tranfusion Centre, Amsterdam. Subclass analysis was done because for TBMC calculation of IgG3 cases other formulas are used then for other IgG cases. Serum immunoglobulin levels were measured by nephelometry using the Beckmann Immunochemistry system (Beckman Instruments International, Geneva). Bence Jones proteinuria was determined by multiplying the proteinuria (g/24 h) with the percentage light chain obtained with densitometry of agarose electropheresis of concentrated urine.

Clinical staging

Disease stage of the patients was determined according to Durie and Salmon [17]. The criteria for stage III disease ($> 1.2 \times 10^{12}$ myeloma cells/m^2 body surface) are: 1. haemoglobin level < 85 g/l; 2. serum calcium > 3.00 mmol/l; 3. serum myeloma protein level for IgG > 70 g/l, for IgA > 50 g/l and light chain excretion > 12 g/24 h; 4. advanced lytic bone lesions. Substaging A or B depends on normal or

impaired renal function (serum creatinine > 180 μmol/l). One criterion, if caused by the disease, is sufficient.

Calculation of TBMC – Response evaluation

For calculation of TBMC and assessment of response a programmable pocket calculator (the HP-65, Hewlett Packard, Cupertino, California) was used [20]. For the patient with an IgA m-protein TBMC changes were calculated by multiplying the pretreatment TBMC with the percentual change in m-protein level. For the patient with a light-chain myeloma TBMC was not calculated. Response was also evaluated according to the criteria of the Southwest Oncology Group [21]. A complete response was indicated by complete disappearance of the m-protein. Partial Response, Improvement and No-Response were defined as a decrease in m-protein level of 75-90%, 50-75%, 0-50%, respectively. Progressive disease was indicated by a significant increase of m-protein and/or signs or symptoms.

Treatment regimen

The treatment regimen comprised intravenous administration of cyclophosphamide (500 mg/m^2 body surface), methylprednisone (600 mg/m^2 body surface), both at day 1, and vindesine (2 mg/m^2 body surface) at day 8. The regimen was repeated every three weeks (day 1 = day 22 etc). In responding patients the treatment interval was gradually increased to a maximum of 5 weeks.

Patients

Six patients with high tumour mass myeloma entered the study. Clinical data are shown in Table 1. TBMC ranged from 2.30-3.59 $\times$ 10^{12}. All patients had normal renal function. Non-written informed consent was obtained from every patient.

5.3 Results

Serial measurements of m-protein level and TBMC (pat. 1-5), and serial determinations of light-chain excretion (pat. 6) are shown in Fig. 1.

Different types of tumour reductions were observed. Patients 1, 2, 4, and 5 showed a rapid decrease of tumour mass. The lowest tumour mass was reached after the first (pat. 5) or second (pat. 1, 2, 4) chemotherapy course.

Table 1. Clinical data of the patients.

	Patient, age	M-type	Skeletal[1] involvement	Pretreatment M-protein (g/l) TBMC ($\times 10^{12}$)	Nadir M-protein (g/l) TBMC ($\times 10^{12}$)	Maximum decrease (in %) of M-protein and TBMC	Response according to SWOG criteria	Follow-up[2]
1.	M.M. ♂ 65	A-K	lesions in skull, l+r humerus, fractures C4, Th 10 diffuse osteopenia	48.2 3.03	17.0 1.09	64.7 64.0	Improvement	alive 42 wk+, progressive disease after 6 × CES, stable on rescue therapy.
2.	A.H-R ♀ 64	G3-K	lesions in skull left femur, C5, L5 large defect in pelvis	71.9 2.93	6.0 0.08	91.7 97.3	Partial Response	alive at 48 wk +, KPS 90, M-protein level stable 6–8 g/l
3.	W.V. ♂ 36	G1-K	lesions in Th I, 7, 8 10, 11, L5 fractures C1, 3, 5 + multiple ribs	113.0 3.59	62.5 1.99	44.7 44.6	No-Respons	alive 54 wk +, KPS 40→90
4.	H.P.de G. ♀ 65	G1-K	diffuse osteopenia fracture L I, 2, 3	67.6 2.37	36.7 1.11	45.7 53.1	No-Respons	alive 61 wk+, progressive disease after 6 × CES, stable on rescue therapy.
5.	H.N. ♂ 73	G1-λ	diffuse osteopenia lesions left humerus and pelvis fracture Th 6, 7	58.3 2.30	20.0 0.6	65.7 73.9	Improvement	died in wk 17 of pneumonia after paraplegia due to spinal cord compression at Th 5.
6.	H.de W. ♂ 56	BJ-K	lesions in skull + left humerus fracture Th 4, 6	9.4 g/24 h light chain excretion	1.8 g/24 h light chain excretion	80.9	Partial Respons	alive 66 wk +, KPS 50 → 90

1. C, Th, and L indicate cervical, thoracic and lumbar vertebrae;
2. KPS = Karnofsky Performance Score.

According to SWOG criteria (Table 1) pat. 2 showed a Partial Response, pat. 1 and 5 showed an Improvement while pat. 4 showed No Response, although her TBMC decreased more than 50%. In contrast, pat. 3 and 6 showed a gradual decrease of TBMC (Fig. 1). Pat. 3 showed No Response and pat. 6 a Partial Response. After initial TBMC reduction pat. 1 and 4 showed a rapid increase of TBMC together with clinical symptoms. They were then treated with a four-drug regimen including adriamycin which resulted in a stable disease in pat. 1 and a gradual decrease of TBMC in pat. 4. Pat. 2, 3 and 6 are still treated with CES and they are in satisfactory clinical condition (Fig. 1/Table 1). Pat. 5 died of pneumonia, three weeks after the occurrence of a paraplegia, due to spinal cord compression at the level of the 5th thoracic vertebra, caused by an extraosseous growing plasma cell mass.

Toxicity of the CES regimen was negligible. Nausea occurred sporadically after the cyclophosphamide administration. Hair loss was absent or minimal in all patients. Moderate leukopenia (nadir $2.1 \times 10^9/l$) was observed in all patients, but only after the first two or three chemotherapy courses. Dose adjustments were never necessary.

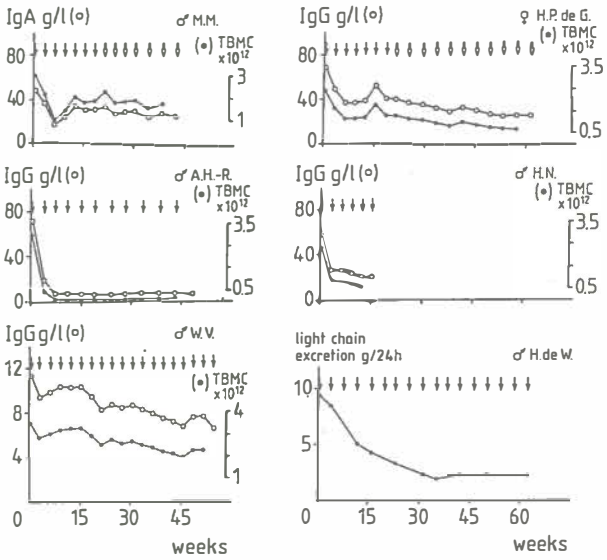

Fig. 1. Serial determinations of serum M-protein and TBMC. For patient 6 only light chain excretion is indicated. Patient H.N. died at 17 weeks.
↓ indicates CES course; ⌟⌟ indicates rescue therapy.

5.4 Discussion

Six patients with a stage IIIA multiple myeloma were treated with a regimen which was based on several reports on cytokinetic data in human myeloma.

Since the results of this pilot study did not fullfill our expectations we decided to change the regimen and report on our experiences with these six patients.

Our first objective (to induce a profound reduction of TBMC) was not reached, since 2 of the 6 patients were non-responders according to the SWOG criteria. Nevertheless, when the responses are compared with the insignificant toxicity of CES, it seems justified to explore the effects of this regimen in a larger number of patients. It should be possible to increase the dose of the cyclophosphamide and/or vindesine until a moderate toxicity is reached.

We also failed our second goal (preventing a relapse) since two of the six patients showed a relapse quite soon after initial tumour reduction. The three types of response (rapid tumour reduction with plateau phase, rapid tumour reduction with rapid relapse, and gradual tumour reduction) observed in the present study, were also observed by Durie [6]. These patients were treated with different drug regimens [22]. It is interesting that these different modes of response were not abolished by a regimen (CES) which was partly devised on the basis of the data presented by Durie[7]. Therefore further investigation into the basis of these different response types is warranted.

The differences in response indicate that it may be important to obtain cell behaviour data in every individual patient. These data should then be combined with data on individual tumour-drug sensitivity testing, before a rational and presumably effective therapy can be instituted.

References

1. Durie BGM, Salmon SE. The current status and future prospects of treatment for multiple myeloma. In: Salmon SE (ed), Clinics in Haematology, Vol 11, no 1, 181-210, W B Saunders Company Ltd, London, 1982.
2. Bonnet JD. The management of multiple myeloma and related disorders. In: Carter SK, Goldstein E, Livingstone R B (eds): Principles of cancer treatment, McGraw-Hill, New York, 1982.
3. Drewinko B, Alexanian R, Boyer H, Barlogie B, Rubinow I. The growth fraction of multiple myeloma. Blood 1981, 57, 333-338.
4. Salmon SE. Expansion of growth fraction in multiple myeloma with alkylating agents. Blood 1975, 45, 119-129.
5. Drewinko B, Brown BW, Humphrey R, Alexanian R. Effect of chemotherapy on the labelling index of myeloma cells. Cancer 1974, 34, 526-531.

6. Durie BGM, Russell DH, Salmon SE. Reappraisal of plateau phase in myeloma. The lancet 1980, ii, 65-68.
7. Durie BGM, Salmon SE, Moon TE. Pretreatment tumour mass, cell kinetics and prognosis in multiple myeloma. Blood 1980, 55, 364-372.
8. Alexanian R, Balcerzak S, Bonnet JD, Gehan EA, Haut A, Hewlett JS, Monto RW. Prognostic factors in multiple myeloma. Cancer 1975, 36, 1192-1201.
9. Karp JE, Burke PJ, Humphrey RL. Induction of serum stimulation and plasma cell proliferation during chemotherapy of multiple myeloma. Blood 1977, 49, 925-934.
10. Karp JE, Humphrey RL, Burke PJ. Timed sequential chemotherapy of cytoxan-refractory multiple myeloma with cytoxan and adriamycin based on induced tumour proliferation. Blood 1981, 57, 468-475.
11. Rivers SL, Patno ME. Cyclophosphamide vs melphalan in treatment of plasma cell myeloma. JAMA 1969, 207, 1328-1334.
12. Cuckle H, Galton DAG, Peto R, Paul E, Gilham E. Report on the second myelomatosis trial after five years of follow-up. Br J Cancer 1980, 42, 813-840.
13. Houwen B, Ockhuizen Th, Marrink J, Nieweg HO. Vindesine therapy in melphalan-resistant multiple myeloma. Eur J Cancer 1981, 17, 227-232.
14. van Dobbenburgh OA, Houwen B, Halie MR, Marrink J, Ockhuizen Th, Nieweg HO. Progress report on vindesine treatment of melphalan resistant myeloma. Eur J Cancer Clin Oncol 1983, 19, 861-862.
15. Riccardi A, Merlini G, Montecucco CM. Treatment of multiple myeloma with vincristine. Acta Haemat 1980, 61, 176-178.
16. Salmon SE, Shadduck RK, Schilling A. Intermittent high-dose prednisone (NSL-10023) therapy for multiple myeloma. Cancer Chemother Rep 1967, 51, 179-187.
17. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 1975, 36, 842-845.
18. Durie BGM. Kinetic studies in myeloma and their implications for treatment. In: Karter SK, Goldstein E, Livingstone RB (eds): Principles of cancer treatment, McGraw-Hill, New York 1982.
19. Ouchterlony O. Diffusion-in-gel methods for immunological analysis. In: Kallos P (ed), Progress in allergy, Vol 5, page 1-78, 1958, Karger Basel/ New York.
20. Salmon SE, Wampler SB. Multiple myeloma: Quantitative staging and assessment of response with a programmable pocket calculator. Blood 1977, 49, 379-389.
21. Bonnet J, Alexanian R, Salmon SE, Bottomley R, Haut A, Amare M, Dixon D. Vincristine, BCNU, doxorubicin and prednisone (VBAP) combination in the treatment of relapsing multiple myeloma: a Southwest Oncology Group study. Cancer Treat Rep 1977, 66, 1267-1271.
22. Alexanian R, Salmon SE, Bonnet J, Haut A, Weick J. Combination chemotherapy for multiple myeloma. Cancer 1977, 40, 2765-2771.

Chapter 6

Plasma spermidine concentrations as early indication of response to therapy in human myeloma

6.1 Introduction

Measurements of polyamines, mainly spermidine and putrescine, have been shown to be useful in experimental research especially concerning tumour growth and response to treatment. Cell death is reflected in an increase of the spermidine concentrations in biological fluids – for example, urine, serum, or cerebrospinal fluid – possibly because spermidine is released by the dying cell [1,2].

In recent years a considerable amount of work has been done to establish practical clinical utility for polyamine measurements. In 1975 Russell et al. showed that an increase of spermidine excretion in urine after cytostatic treatment correlated with clinical response [3] in solid and haematological tumours. These results were extended and confirmed [4]. Since 1977, however, the only clinical application for measurement of polyamines has been monitoring of medulloblastoma by measuring putrescine concentrations in cerebrospinal fluid [1].

One of the possible reasons for a decline in interest [1] is, that most polyamine determinations were done on 24-hour urine. Samples are cumbersome to obtain and collection may be incomplete. Recently, a radioimmunoassay for spermidine measurement in plasma became available to us, and, prompted by the studies of Russell et al. [3,4], we investigated the possibility of drug sensitivity testing in vivo.

In this chapter we present the results of plasma spermidine measurements before and after an injection of vindesine in patients with melphalan-resistant multiple myeloma. The correlation of the spermidine concentration with the clinical response is investigated.

6.2 Patients and methods

Spermidine concentrations were measured by a radioimmunoassay [5]. Antibodies to spermidine were raised in rabbits by immunisation with spermidine covalently bound to bovine thyroglobulin. Plasma spermidine concentrations were estimated by using a double-antibody technique. Only free spermidine is measured, the plasma is not

pretreated by acid hydrolysis. Cross reaction with spermine or putrescine is less than 1%. Spermidine concentrations are expressed in nmol/l. Venous blood samples were taken in heparinised vacuum glass tubes. Directly after venepuncture the blood was centrifuged (3000 rpm for 10 min) and plasma was frozen and stored at -20 °C. Haemolytic samples were discarded. The spermidine assay took place within 14 days after sampling.

Patients

All patients were treated with vindesine and prednisone for multiple myeloma resistant to melphalan/prednisone treatment. The preliminary results of this 'rescue' therapy have been published [6,7].

Vindesine was given as an intravenous bolus injection (2 mg/m^2) at weekly intervals for three consecutive weeks, followed by a three week therapy-free interval. After each vindesine injection prednisone was given orally in daily doses of 100 mg for 5 days. Evaluation of response took place after three months of treatment, response was measured according to the Southwest Oncology Group (SWOG)-criteria [8] which differentiate between 'responders', 'improved patients' and 'non-responders'. In this paper the improved patients as well as the responders are called 'responders'. All patients were classified according to Durie [9] as stage III disease (high tumour load).

Protocol

Patients were admitted to hospital for their first vindesine injection. This injection was not followed by prednisone because of the probable stimulating effect of prednisone on polyamine metabolism [10]. The baseline plasma sample was taken at 0800 hour and vindesine was administered immediately afterwards. Further samples were taken at 2, 4, 6 and 8 h after the vindesine injection and daily at 0800 hour for the next five or six days. Patients were free from bacterial infections. As controls, samples were taken from 12 healthy volunteers at 0800, 1100, 1300 and 1600 hour.

Statistics

For correlation of response and spermidine concentration the 2×2 contingency tables were used.

6.3 Results

Pretreatment values of the patients are shown in Fig. 1 (range 50-920 nmol/l, mean 290 nmol/l) and they are compared with the 0800 hour values of the 12 controls (range 90-550, mean 270.5 nmol/l). Although two of the patients had very high pretreatment values it seems that these initial values do not discriminate between patients and controls, nor do they discriminate between responders to treatment and non-responders (open vs closed circles, Fig. 1).
Spermidine concentrations of the 12 healthy volunteers over the day are shown in Fig. 2. Values are expressed as a percentage of the 0800 hour sample. A diurnal fluctuation of ± 50% was observed.

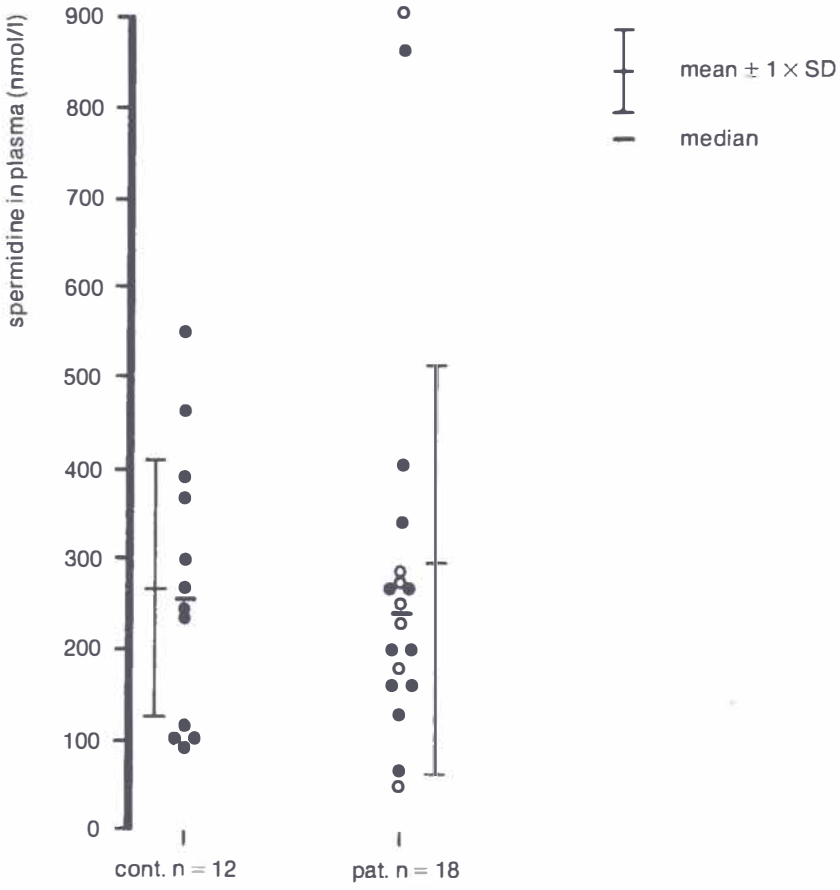

Fig. 1. Base line plasma spermidine values of the controls and patients. Open circles indicate responding patients.

The spermidine values, after the vindesine injection expressed as a percentage of the pretreatment value, are shown in Fig. 3. Because of the diurnal fluctuation observed in the controls we considered that a change in spermidine values of $> 100\%$ in the patients, after treatment, was significant. The patients were classified according to (a) response versus non-response to treatment and (b) significant versus the non-significant rise of spermidine concentrations.

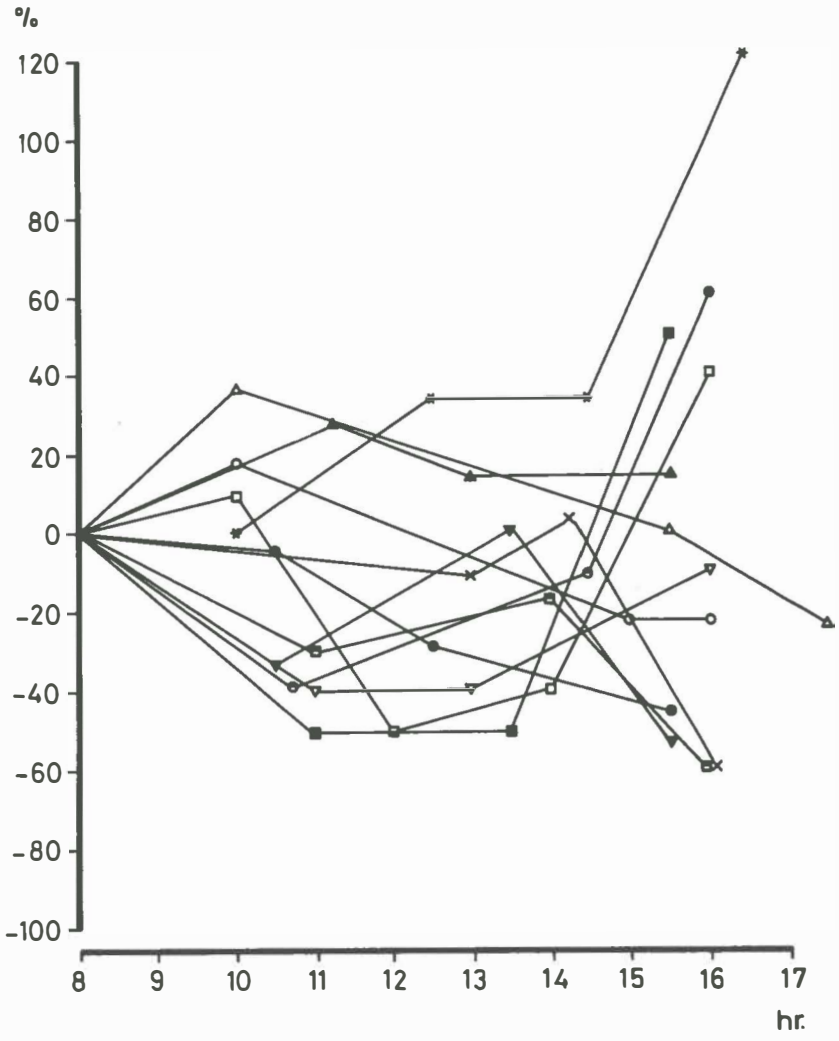

Fig. 2. Plasma spermidine values of 12 healthy volunteers. Values are expressed as a percentage of the 0800 h value.
* One curve starts at 10.00 h.

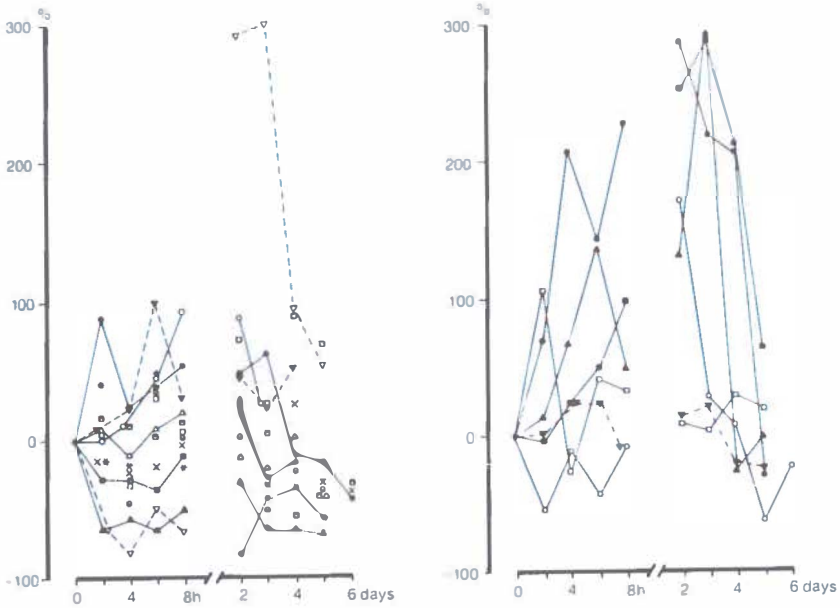

Fig. 3. Plasma spermidine values after a vindesine injection (0 h) expressed as a percentage of the base line (0 h) value.
Non-responders to therapy, left; Responders to therapy, right.
Dashed lines indicate two non-responders with a significant spermidine rise (left) and one responder without a significant rise (right).

Five of the six responders showed a spermidine rise of $> 100\%$ while ten of the twelve non-responders did not show a significant rise (Table). The correlation between response/non-response and significant/non-significant spermidine rise was statistically significant ($p < 0.05$). From the same figures a decision matrix was calculated (Table). Sensitivity and specificity were both 83%. The predictive value of a significant spermidine rise was 71%; the predictive value of absence of a spermidine rise was 91%.

6.4 Discussion

Recently a radioimmunoassay for plasma spermidine concentration became available to us. In patients with melphalan-resistant multiple myeloma who were treated with vindesine and prednisone we studied plasma spermidine concentrations after vindesine administration. Although all patients had Stage III disease, only two patients showed extremely high pretreatment values, probably indicating increased

spontaneous cell death. This could indicate different tumour kinetics in individual patients and is in accordance with the view of Durie [11] who demonstrated different tumour doubling times within one group of patients with the same stage of disease.

The spermidine concentrations of the responding patients showed a rise within 24 hour indicating cell death quite soon after the vindesine administration, which agrees with in vitro experiments [12]. It also correlates with our clinical observation of rapidly decreasing skeletal pain in several patients. Both observations suggest that plasma spermidine concentrations can be used for research on drug and tumour kinetics.

Table 1. Correlation between response/non-response and spermidine rise/non-spermidine rise ($p < 0.05$).

	Significant spermidine rise		
	+	-	
Response	5	1	sensitivity[1] 83%
Non-response	2	10	specificity[2] 83%
Total	7	11	
	PV pos.[3] 71%	PV neg.[4] 91%	

1. sensitivity: true positive ratio;
2. specificity: true negative ratio;
3. PV pos.: predictive value of a positive test result;
4. PV neg.: predictive value of a negative test result.

Pretreatment spermidine concentrations did not distinguish patients from controls, nor between those who responded to treatment and those who did not. A statistically significant correlation was shown between a significant rise of the plasma spermidine value (after drug administration) and a clinical response. With the spermidine values obtained in this particular protocol, response and especially non-response could be predicted with reasonable accuracy (decision matrix, see Results). If this (or a similar) protocol could be used to predict response or non-response to other cytostatic drugs, the possibility arises to test tumour drug sensitivity in vivo in the individual patient. In analogy to tumour drug sensitivity testing in vitro (tumour stem cell assay) treatment could then be selected on an individual basis.

This could be especially useful in malignancies in which response or non-response to treatment is not always readily apparent, as in e.g. multiple myeloma, where the prolonged half life of the m-protein may delay response evaluation.

The radioimmunoassay for spermidine is a rapid method, so drug sensitivity testing for one or more drugs should enable the physician

to select proper treatment shortly after his decision to treat the patient.

Since the present study is limited to a small group of patients, in which only one drug is tested for one disease, further studies are needed to confirm the usefulness of plasma spermidine measurements in tumour drug sensitivity testing.

References

1. Marton LJ, Seidenfeld J. Approaches to the study of polyamines as cancer markers. In: Morris DR, Marton LJ, eds. Polyamines in biology and medicine. Vol. 8 in The Biochemistry of disease. M.Dekker 1981, 337-348.
2. Milano G, Schneider M, Cassuto JP, et al. Polyamines: comments on clinical utility in cancer. A review. Tumor Diagnostik 1980, 3, 121-125.
3. Russell DH, Durie BGM, Salmon SE. Polyamines as predictors of success and failure in cancer chemotherapy. Lancet 1975, ii, 797-799.
4. Durie BGM, Salmon SE, Russell DH. Polyamines as markers of response and disease activity in cancer chemotherapy. Cancer Res 1977, 37, 214-221.
5. Jurjens H, Bijzet J, Woldring MG. Radioimmunoassay of spermidine in extracted plasma. Submitted.
6. Houwen B, Ockhuizen Th, Marrink J, Nieweg HO. Vindesine therapy in melphalan-resistant multiple myeloma. Eur J Cancer 1981, 17, 227-232.
7. Van Dobbenburgh OA, Houwen B, Halie MR, Marrink J, Ockhuizen Th, Nieweg HO. Progress report on vindesine treatment of melphalan resistant multiple myeloma. Europ J Cancer Clin Oncol 1983, 19, 861-862.
8. Alexanian R, Bonnet J, Gehan E, Haut A, Lane M, Monto R, Wilson H. Combination chemotherapy for multiple myeloma. Cancer 1975, 36, 842-854.
9. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Cancer 1975, 36, 842-854.
10. Jänne J, Pösö H, Raina A. Polyamines in rapid growth and cancer. Biochem Biophys Acta 1978, 473, 241-293.
11. Durie BGM. Staging and kinetics of multiple myeloma. In: Clinics in hematology. Philadelphia: WB Saunders. 1982, 3-18.
12. Hill BT, Whelan RDH. Comparative effects of vincristine and vindesine on cell cycle kinetics in vitro. Cancer Treatment Rev 1980, 7(suppl), 5-15.

Summary

Chapter 1 consists of a review of the literature in which the following aspects of multiple myeloma are discussed:

a) history
b) differential diagnosis of a monoclonal immunoglobulin
c) symptomatology
d) criteria for the diagnosis
e) staging of the disease
f) treatment
g) prognosis
h) cytokinetic data

The background and the purpose of this thesis: the improvement of the management of this disease by the use of the new drug vindesine, are discussed.

In Chapter 2 an investigation into the benefits and toxicity of a treatment regimen consisting of vindesine and prednisone is described. Vindesine (desacetyl-vinblastine amide sulphate, Eldisine®) is a vinca alkaloid differing from the two earlier drugs vincristine and vinblastine. It was used in this phase-II trial because it has less neurotoxic potential than vincristine. The regimen was applied in 40 patients with multiple myeloma resistant to melphalan-prednisone. Thirty-four of these patients could be evaluated. Five patients showed a 'partial response' (more than 75% reduction of m-protein level) and four showed an 'improvement' (more than 50% reduction). Eighteen patients showed a m-protein reduction between 0 and 50% and in seven patients disease progression occurred without reaction to therapy.

Treatment was continued in the nine responding patients. The initial response was short-lasting: four patients showed progressive disease after 14 to 30 weeks. Three patients died of infection, while no signs of renewed tumour activity were present. One patient died of an unknown cause and only one patient is in long term remission. Other side effects were alopecia (15% of the patients) and fingertip paraes-

thesias (50% of the patients). Leukopenia was infrequently observed and never severe.

These results indicate a potential therapeutic effect of vindesine at least in some cases of multiple myeloma. However, the combination with prednisone as used in the present study does not seem promising. Additional investigation of the effects of vindesine – with or without other drugs – seems necessary before a definitive evaluation of vindesine in this disease can be made.

Chapter 3 presents the results of an investigation into the effectivity and toxicity of a new combination therapy for patients with melphalan-resistant multiple myeloma.

This regimen – based on the foregoing observations on vindesine – consisted of cyclophosphamide 500 mg/ m^2 intravenously on day 1 (or orally divided over day 1-5), adriamycin 25 mg/ m^2 intravenously on day 1, prednisone 60 mg/ m^2 orally on day 1-5 and vindesine (Eldisine®) 2 mg/ m^2 intravenously on day 1. The regimen ('CAPE') was administered every three to four weeks.

Nine of the twenty patients that could be evaluated had been treated with other second-line regimens before they received CAPE. The response criteria were the same as in Chapter 2. Two 'partial responses' and two 'improvements' were observed. Eleven patients showed 'stable disease' while in five patients tumour progression continued. Median survival calculated from the start of CAPE was thirteen months. At the moment of evaluation fourteen of 22 patients were alive.

Adverse effects were nausea, vomiting and alopecia in the majority of the patients. In two patients congestive heart failure developed, which may have been induced by adriamycin (cumulative doses of 560 and 300 mg/ m^2 in these patients). Bone marrow toxicity resulted in mild to severe leukopenia in eleven patients and in moderate to severe trombocytopenia in three patients. Two patients died while they were leukopenic; both were already leukopenic before CAPE was started.

The management of patients with melphalan-resistant multiple myeloma is cumbersome and rarely satisfactory. These patients frequently have bone marrow insufficiency due to previous chemotherapy and/or replacement by plasma cells. At present there is no generally accepted treatment (see also Chapter 1). Further use of CAPE in a larger patient population seems appropriate so that the indication for this or a similar combination can be established.

In Chapter 4 the value of β2-microglobulin (β2-m) determinations in serum in the management of patients with multiple myeloma is

investigated. In a retrospective analysis of 87 patients it was shown that serum β2-m levels at the time of diagnosis did not correlate with the disease stages of Durie and Salmon. β2-m serum levels also did not discriminate between patients with MGUS (n = 85) and patients with multiple myeloma stage IA.

Initial β2-m levels correlated significantly with haemoglobin levels, serum calcium levels, the IgG levels in IgG myeloma, the TBMC (calculated tumour cell mass), the Karnofsky Performance Score and serum creatinine levels. The r-values for these correlations where low, except for the correlation with creatinine (r = 0.68). After correction for renal function (β2-m M/CTD) correlation remained significant only for haemoglobin and IgG levels. The r-values were low.

For survival a significant correlation with the initial β2-m serum level was found. Patients with an initial β2-m below 2.9 μg/ml lived longer than patients with a β2-m level above 2.9 μg/ml (respective median survival times were 65 and 25 months). In a multivariate analysis with renal function, age, TBMC, serum calcium, haemoglobin level and β2-m only the serum creatinine, the TBMC and the age had independent prognostic value. From data in the literature it is known that serum β2-m levels are strongly influenced by the kidney. Thus, the prognostic value of β2-m reflects that of the renal function.

The relation between changes in β2-m levels and changes in TBMC after six or twelve months of treatment was investigated. No correlation between these parameters was found. It is concluded that β2-m levels correlate only minimally with the tumour mass while they do not have additional value for the prediction of the prognosis.

Chapter 5 contains the results of a pilot study in which 6 patients with multiple myeloma stage IIIA (high tumour load) were treated with an experimental chemotherapy combination. This combination had been designed on the basis of cytokinetic data from the literature. The regimen consisted of cyclophosphamide 500 mg/m^2 and methylprednisolon 600 mg/m^2 on day 1 followed by vindesine 2 mg/m^2 on day 8.

Two patients showed a rapid response and remained in remission although one of them died with a paraplegia due to an intraspinal growing plasmacell mass. Two patients showed a gradual response and remained in remission in excellent condition. Finally, two patients showed a rapid response followed by a rapid return of tumour activity.

Thus, the rapid return of disease activity which had previously been reported in 'rapid responders', could not be prevented. Since this was the goal of this drug combination, no further patients were treated. The toxicity was minimal, allowing a dose escalation, which can probably result in a more effective regimen.

The possibility of testing drug sensitivity of the tumour in vivo was discussed in Chapter 6. In eighteen patients with melphalan-resistant multiple myeloma spermidine levels have been measured by radioimmunoassay, before and after an intravenous vindesine administration. The patients were then treated with the vindesine-prednisone combination described in Chapter 2. Afterwards the clinical response was compared with the rise of the spermidine plasma level. Six patients showed a tumour reduction and in five of them a significant rise of the plasma spermidine level had occurred after the vindesine injection.

In ten out of eleven non-responders no spermidine rise was measured. Base line plasma spermidine levels did not discriminate between controls and patients and also not between responsers and non-responders.

The significant correlation between a rise in plasma spermidine level and a therapeutic response point to the feasibility of tumour-drug sensitivity testing in vivo. This study concerned only patients with melphalan-resistant myeloma. Additional studies in patients with other malignant diseases should be performed to see whether this method is useful for treatment selection individual patients.

Samenvatting

Hoofdstuk 1 geeft een kort overzicht van de literatuur betreffende de volgende facetten van de ziekte van Kahler (multipel myeloom):

a) historie
b) differentiële diagnose van een monoclonaal immuunglobuline
c) symptomatologie
d) diagnostische criteria
e) indeling in verschillende stadia
f) behandeling
g) prognose
h) cytokinetische aspecten

Tevens worden de achtergrond en de doelstelling van het in dit proefschrift beschreven onderzoek besproken. Er werd gestreefd naar verbetering van de behandeling van deze ziekte, met name door toepassing van het vindesine.

In Hoofdstuk 2 wordt een onderzoek beschreven naar de effectiviteit en toxiciteit van een behandelingsschema bestaande uit vindesine en prednison. Vindesine (desacetyl-vinblastine amide sulfaat, Eldisine®) is een recent ontwikkeld vinca alkaloide naast het langer bekende tweetal: vincristine en vinblastine. De neurotoxiciteit van vindesine is aanmerkelijk minder dan van vincristine. Dit schema werd toegepast bij 40 patienten met multipel myeloom, waarbij zich resistentie had ontwikkeld tegen de behandeling met de combinatie melfalan-prednison. Bij 34 van hen kon de reactie op de therapie worden beoordeeld. Vijf patienten toonden een 'partiële response' (meer dan 75% daling van de paraproteïne spiegel) en vier patienten toonden een 'verbetering' (meer dan 50% daling). Achttien patienten toonden een paraproteïne spiegel reductie tussen nul en 50% en in zeven patienten was er sprake van progressie zonder enige op de behandeling.

Bij de negen patienten met duidelijke reductie van de tumor werd de behandeling voortgezet. De aanvankelijk goede reactie bleek van korte duur: vier patienten toonden na veertien tot 30 weken duidelijke tumor progressie. Drie patienten overleden aan een infectie, overigens

zonder dat er tekenen van hernieuwde ziekte activiteit waren. Eén patient stierf zonder dat de oorzaak kon worden achterhaald en slecht één patient is in langdurige remissie. De bijwerkingen bestonden uit haaruitval (15% van de patienten) en paraesthesiën in de vingertoppen (50% van de patienten). Leucopenie trad slechts zelden op en was nooit van ernstige aard.

Deze resultaten wijzen erop dat vindesine een werkzaam middel is in de behandeling van de ziekte van Kahler. De in deze studie gehanteerde combinatie met prednison lijkt echter weinig aantrekkelijk. Verder onderzoek naar de werkzaamheid van vindesine – al dan niet in combinatie met andere middelen – lijkt aangewezen.

In Hoofdstuk 3 worden de resultaten beschreven van een onderzoek naar de effectiviteit en toxiciteit van een nieuw combinatie schema, dat werd gebruikt als tweede lijns ('rescue') behandeling bij patienten met melfalan-resistent multipel myeloom.

Het schema – ten dele gebaseerd op de ervaringen opgedaan met vindesine/prednison – bestond uit cyclofosfamide 500 mg/m^2 intraveneus op dag 1 (of oraal, verdeeld over dag 1 t/m 5), adriamycine 25 mg/m^2 intraveneus op dag 1, prednison 60 mg/m^2 per os op dag 1 t/m 5 en vindesine (Eldisine®) 2 mg/m^2 intraveneus op dag 1. Deze combinatie van middelen ('CAPE') werd eens per drie of vier weken toegediend.

Van de twintig patienten die beoordeeld konden worden waren er negen reeds met een andere 'rescue' combinatie behandeld voordat zij CAPE kregen toegediend. De criteria voor het beoordelen van het effect waren dezelfde als vermeld in Hoofdstuk 2. Er waren twee 'partiële responses' en twee 'verbeteringen'. Bij elf patienten was er stabilisering van de ziekte terwijl bij vijf geen enkele verbetering werd waargenomen. De mediane overleving gerekend vanaf het begin van de CAPE-behandeling was dertien maanden. Op het moment van evaluatie waren veertien van de 22 patienten in leven.

De bijwerkingen bestonden uit misselijkheid, overgeven en haaruitval in de meerderheid van de patienten. Twee patienten ontwikkelden decompensatio cordis, welke veroorzaakt zou kunnen zijn door adriamycine (cumulatieve dosering 560 en 300 mg/m^2 in deze patienten). Beenmerg remming uitte zich in lichte tot ernstige leucopenie in elf patienten en in matige tot ernstige trombocytopenie in drie patienten. Twee patienten overleden in een leucopenische fase, maar beiden hadden reeds leucopenie voordat met CAPE was begonnen.

De behandeling van patienten met melfalan-resistent multipel myeloom is gecompliceerd en vaak ondankbaar. De patienten hebben meestal een insufficiente haemopoiese ten gevolge van verdringing

en/of voorafgaande chemotherapie. Er bestaat tot op heden geen algemeen geaccepteerde 'rescue' therapie (zie ook Hoofdstuk 1). Toepassing van het CAPE-schema in een grotere groep patienten lijkt gewenst opdat de plaats in de behandeling definitief kan worden vastgesteld.

In Hoofdstuk 4 wordt een onderzoek beschreven naar het nut van β2-microglobuline (β2-m) bepalingen in serum voor de behandeling van patienten met multipel myeloom. In een retrospectief onderzoek met 87 patienten bleek dat de serum β2-m spiegel bij het stellen van de diagnose slecht correleerde met de stadia volgens Durie en Salmon. Evenmin kon op grond van deze spiegel onderscheid worden gemaakt tussen patienten met benigne monoclonale gammopathie (n = 85) en patienten met multipel myeloom stadium IA. Het serum β2-m gehalte toonde verder significante correlaties met het haemoglobine, het serum calcium, de IgG spiegel in IgG-myelomen, de TBMC (berekende tumor massa), de Karnofsky Performance Score en het serum creatinine. De r-waarden voor deze correlaties waren laag, behalve voor die met het serum creatinine (0,68). Een voor de nierfunctie gecorrigeerd β2-m gehalte correleerde nog slechts significant met het haemoglobine gehalte en met de IgG spiegel. Ook hier waren de r-waarden laag.

Vooral voor de overlevingsduur werd een duidelijke correlatie met het β2-m gehalte gevonden. Patienten met een initiele β2-m spiegel lager dan 2,9 μg/ml leefden significant langer dan patienten met een spiegel boven de 2,9 μg/ml (mediane overleving respectievelijk 65 en 25 maanden). In een multivariant analyse met als parameters nierfunctie, leeftijd, TBMC, serum calcium, haemoglobine en β2-m bleken echter alleen het serum creatinine gehalte, de TBMC en de leeftijd van belang. Uit de literatuur is bekend dat serum β2-m spiegels sterk van de nierfunctie afhankelijk zijn. De prognostische waarde van β2-m spiegels weerspiegelt derhalve die van de nierfunctie.

Verder werd nog gekeken naar de relatie tussen verandering in β2-m spiegels en verandering in TBMC na zes of twaalf maanden behandeling. Er bestond tussen beide parameters geen enkele correlatie. Op grond van deze gegevens wordt geconcludeerd dat de β2-m spiegel slecht correleert met de tumor massa en geen additionele waarde heeft voor het beoordelen van de prognose.

In Hoofdstuk 5 wordt een 'pilot study' beschreven waarin zes patienten met de ziekte van Kahler met een grote hoeveelheid tumor werden behandeld met een experimenteel chemotherapie regiem. Dit regiem, dat werd samengesteld op grond van literatuurgegevens over

celkinetiek van het multipel myeloom, bestond uit cyclofosfamide 500 mg/ m^2 en solumedrol 600 mg/ m^2 op dag 1, gevolgd door vindesine 2 mg/ m^2 op dag 8. Dit schema werd om de drie weken herhaald.

Twee patienten toonden een snelle response en bleven in remissie hoewel een van deze patienten overleed aan een dwarslaesie ten gevolge van een intraspinaal groeiende myeloom haard. Twee patienten toonden langzame tumor reductie en bleven daarna in remissie. De laatste twee patienten toonden een snelle response gevolgd door een snelle relapse.

Geconcludeerd wordt dat dit regiem therapeutische mogelijkheden biedt, maar dat de snel terugkerende ziekteactiviteit, zoals die in de literatuur word beschreven bij 'snelle responders', niet werd voorkomen. Aangezien de toxiciteit gering was lijkt het mogelijk het regiem qua dosering te intensiveren. Dit zou onderwerp kunnen zijn voor een volgende studie.

Hoofdstuk 6 vermeldt de resultaten van een onderzoek naar de mogelijkheid van het voorspellen van het effect van een cytostaticum op de tumor. Hiertoe werden bij patienten met melfalan-resistent myeloom plasma spermidine spiegels gemeten, voor en na intraveneuze toediening van vindesine. De hierop volgende behandeling bestond uit de combinatie vindesine en prednison zoals beschreven in Hoofdstuk 2. De al dan niet optredende stijging van spermidine in het plasma werd vergeleken met de later optredende tumor respons.

Van de achttien patienten toonden zes een reductie van de tumor. Bij vijf van hen was er een significante stijging van de spermidine spiegel waargenomen na de eerste injectie van vindesine. Bij tien van de elf niet reagerende patienten werd geen stijging gemeten.

Op grond van deze gegevens lijkt het mogelijk – in vivo – de gevoeligheid van een tumor voor cytostatica te testen. In dit onderzoek betreft het patienten met een voor een of meerdere cytostatica resistent myeloom. Verder onderzoek bij patienten met andere maligne ziekten zal moeten uitwijzen of deze methode inderdaad aanwijzingen geeft over de gevoeligheid van de tumor voor een medicament en of dit van practisch nut kan zijn bij het selecteren van een behandeling voor de individuele patient.